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COPPER REQUIREMENT OF WHEAT (*Triticum aestivum* L.) CULTIVARS

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BY

JAMES OTIENO OWUOCHE

DEGREE: **Master of Science.**

YEAR THIS DEGREE GRANTED: **1992.**

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

IN

PLANT BREEDING

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

FALL 1992

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled COPPER REQUREMENT OF WHEAT (*Triticum aestivum* L.) CULTIVARS submitted by JAMES OTIENO OWUOCHE in partial fulfillment of the requirements for degree of MASTER OF SCIENCE in PLANT BREEDING.

....
Dr. K.G. Briggs.

Gregory Taylor
....
Dr. G.J. Taylor.

....
Dr. W.H. Vanden Born.

....
D.C. Penney

Date....14/7/92.......

ABSTRACT

Copper (Cu) is one of the most limiting micronutrients in wheat grain production (*Triticum aestivum* L.). The objectives of these studies were to (i) determine the response of eight Canadian spring wheat cultivars to Cu and to determine Cu concentration in the youngest fully emerged leaves (YFEL) in the field, and (ii) determine the Cu-efficiency of 5A/5RL wheat-rye translocated lines and nine wheat cultivars on Cu-deficient soil in the greenhouse. A two year field study was conducted at Stony Plain, Central Alberta (53° 02' N - 114° 00' W). The soil at the experimental site had a pH of 5.4, and 0.48 μg Cu g^{-1} soil. Eight cultivars were tested in two Cu treatments (12.2 kg ha^{-1} Cu applied, and no Cu applied). The YFEL were analysed for Cu using atomic absorption spectrophotometry. A dose response experiment with four wheat cultivars and nine levels of Cu were also conducted. Twelve genotypes were evaluated in the greenhouse in two Cu treatments (145 $\mu g\ kg^{-1}$ soil Cu applied, and no copper applied). In the field trial, results indicated significant differences ($P \leq 0.01$) due to cultivars, copper and year for yield, and for some yield components. A significant ($P \leq 0.05$) cultivar × Cu interaction was observed for grain weight . Year × cultivar × Cu interactions were significant ($P \leq 0.05$) for floret fertility, and for number of grains $spike^{-1}$ on the main stems. Copper treatment significantly ($P \leq 0.05$) increased grain weight, the number of grains $spike^{-1}$, and grain yield on cv. Oslo, Park, Roblin, and Conway. Copper concentrations in YFEL ranged between 2.8 and 5.7 $\mu g\ g^{-1}$ on cvs. Katepwa, Park and Roblin when symptoms first appeared. The level of Cu in the YFEL depend on cultivar, growth stage, year and Cu status in the soil. The cultivar Biggar increased grain yield by 81%, and cv. Oslo by 419% when Cu was supplied at 145 $\mu g\ kg^{-1}$ soil. Significant differences ($P \leq 0.05$) due to the 5A/5RL chromosome translocation were observed for grain yield and some yield components. Differential response to Cu supply was observed between 5A/5RL wheat-rye translocated lines compared to other wheat cultivars for grain yield and for number of grains $spike^{-1}$ of tillers. The 5A/5RL wheat-rye translocated lines showed a Cu-efficiency range of 70-127%.

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CHAPTER 1

1.0.0 LITERATURE REVIEW.

Wheat (*Triticum aestivum L.*) is one of the world's major food sources and as such, is a major source of essential nutrients. Deficiencies of micronutrients like copper (Cu) in crops is often reflected in animals (Eagan, 1975). In order to avoid the transfer of the deficiency problem to man and livestock, it is important for the plants to be supplied with adequate copper. Under field conditions, utilization of copper depends on both environmental factors and genetic potential (Bates 1971).

1.1.0 Environmental factors.

1.1.1 Soil properties and related factors.

Important components of the plants environment include the type of soil, copper availability in the soil, soil pH, the root-soil relationship, the interaction of Cu with other nutrients, and soil moisture regime. Soils high in organic matter (Kubota and Alloway, 1972) and weathered mineral soils (Murphy and Walsh, 1972) have been associated with copper deficiency. In Saskatchewan, the critical level of Cu in the soil for spring wheat (*T. aestivum* L.) has been reported to be 0.4 mg kg^{-1} soil, while that of canola (*Brassica napus* L.) is 0.35 mg Cu kg^{-1} soil, on grey and brownish-grey podzolic soils (Kruger *et al.*, 1985). Critical levels of 0.7 μg g^{-1} soil (Penny *et al.*, 1988) in Alberta, 0.2 μg g^{-1} (Alison and Alloway, 1981) in Britain and 0.6 μg g^{-1} soil in Brazil (Leon, 1990) have also been reported.

1.1.2 Soil and rhizosphere pH

Soil pH influences the solubility, speciation, soil solution concentration, mobility, and availability of Cu for plant roots. Maximum adsorption of Cu occurs at pH 5.5 on soils and soil constituents, but at low or high soil pH values the available Cu has been known to be limited (MacLaren *et al.*, 1973). The pH of the rhizosphere may differ from that in the bulk soil and the increase or decrease of pH is determined by plant factors and by the buffering capacity of the soil. Root-induced pH changes in the rhizosphere have been found to be maximal in bulk soil pH between 5.0 and 6.0 and decrease in magnitude at both lower or higher bulk soil pH (Nye, 1986; Schaller, 1987). Wheat has been shown to be sensitive to Cu-deficiency at all soil pH levels, while triticale (X. *Triticosecale* W.), a hybrid between wheat and rye (*Secale cereale* L.), is tolerant to Cu-deficiency at pH 5.0 but sensitive above pH 8.4 (Susan and Graham, 1981). Generally, Cu-deficiency is influenced by the chemical and physical properties of the soil, by the rhizosphere and by the genotype of the plant (Sauerbeck and Helal, 1990). Under low soil moisture regimes, the response of wheat to applied Cu has been shown to be significantly lower than when moisture was more available (Grundon, 1991).

1.1.3 Interactions of copper with other elements

Interactions between Cu and other nutrients occur when plants are exposed to stresses that enhance low concentration of Cu in the soil. High concentrations of Cu in the soil have been reported to aggravate Zn-deficiency in wheat by increasing growth and by depressing Zn absorption (Chaudhry and Loneragan, 1970). A study by Bowen (1987) on excised roots of rice (*Oryza sativa* L) and tomato (*Lycopersicum esculentum* L.) showed that Zn^{2+} and Cu^{2+} are antagonistic for the absorption. High rates of phosphorous supply to the soil induce Cu-deficiency in subterranean clover (*T. subterranean* L. cv. Seaton Park) and other field crops (Murphy *et al.*, 1981; Reuter *et al.*, 1981b; Clark, 1983). According to these findings, the deficiency was induced due to a high rate of growth which increases the demand for copper. Amendment of Cu-deficient calcareous soil (15% $CaCO_3$, pH 8.6), by using $CuSO_4$, progressively impairs Fe mobilization, although Cu mobilization is enhanced (Zhang *et al.*, 1991). Excessive applications of nitrogen fertilizers to wheat plants induce Cu deficiency by encouraging fast growth and increasing the demand for Cu (Chaudhry and Loneragan, 1970). This finding has been recently confirmed by the work of Brennan (1991b) who reported that application of nitrogen fertilizers on wheat fields with marginal and deficient levels of Cu aggravated Cu-deficiency. However, limited information is available on interactions of Cu with other elements within the plant's systems. Most of the work done on the interactions between Cu and other elements has been in soil systems. Loneragan (1975) suggested that Cu interacts with nitrogen within the plant, and forms complex proteins which are immobile.

1.2.0 Genetic factors.

Wheat (*T. aestivum* L.) and closely related species differ extensively in their uptake, translocation, accumulation and use of copper (Clark, 1983). These differences are mainly genetic, but phenotypic expressions may differ when plants are grown under different environments. Diversity on the basis of Cu uptake, translocation, distribution and use has been observed among genotypes and cultivars within the same plant species (Nambiar, 1976; Graham and Pearce, 1979; Sarc, 1981). Although there has been variability in Cu-efficiency among wheat cultivars, the genetic potential for grain yield improvement in these materials is low. Consequently, wheat breeders have been compelled to look for desirable genes from wheat-related species in order to improve on both Cu-efficiency and grain yield potential.

Rye has been reported to be Cu-efficient (Graham, 1978; Graham and Pearce, 1979) and has been a good source of genetic variability for disease resistance for wheat (Seith *et al.*, 1988; Ren, *et al.*, 1990). Differences between cultivars on the basis of copper nutrition have been reported in cereals (Smilde and Henkens, 1967b; Nambiar *et al.*, 1976; Susan and Graham, 1980). Comparisons of Cu efficiency among rye, hexaploid and octoploid triticale, bread wheat, and durum wheat (*T. durum* L.) have revealed that rye is the most Cu-efficient, followed by hexaploid triticale (Graham and Pearce, 1979).

Susan and Graham (1981) observed a relationship between Cu-efficiency and the hairy peduncle, a morphological character that is present on rye and triticale. The hairy peduncle is controlled by genes that are located on chromosome 5R of rye (McIntosh, 1988). In a study using ^{64}Cu, Graham *et al.*, (1981) reported that rye has the highest rate of Cu absorption in solution culture, followed by triticale and wheat. Differences in copper efficiency in rye have been attributed to the differences in internal requirement for Cu, the architecture of the root system, the density and the influence of the roots on the rhizosphere, the capability to extract copper from Cu-deficient soil, the rate of copper uptake, and to translocation effects (Clark, 1983).

1.3.0 Diagnosis of copper deficiency.

1.3.1 Symptoms.

The use of symptoms as a diagnostic tool may not be accurate, because it is easy to confuse the effects of soil moisture deficit with deficiency symptoms of other elements (e.g. floret sterility caused by boron deficiency (Rerkasem *et al.*, 1991)). When marginal Cu-deficiency situations exist, no symptoms are expressed. In cereals, Cu-deficiency produces several symptoms which are characteristic and markedly similar over a wide range of environments. However, significant grain yield loss up to 20% may occur without visual symptoms (Graham and Nambiar, 1981; Tills and Alloway, 1981). Similar effects of Cu-deficiency have been reported on subterranean clover cv. Seaton Park (Reuter *et al.*, 1981a). Common symptoms of copper deficiency are wilting, and withered twisted tips on the young developing leaf and spike (Gartrell and Pearce, 1979). The young leaves of Cu-deficient plants often do not unfold and their collapse is attributed to the damage of the tissue structure caused by retarded lignification (Bussler, 1981). In barley (*Hordeum vulgare* L.) and wheat (*T. aestivum*), Cu-deficiency has been known to induce proliferation of tillers which then bear malformed spikes or no spike, in severe conditions (Snowball and Robson, 1984). Delayed heading, maturity, and senescence are among the symptoms which occur under moderate to severe Cu deficiency situations (Loneragan *et al.,* 1980; Graham and Nambiar, 1981; Snowball and Robson, 1984). At the reproductive stage, pollen sterility can occur both in wheat and barley (Graham, 1975; Graham and Pearce, 1979; Dell, 1980; Jewell *et al.,* 1988). Melanism, a dark pigmentation on the stem, peduncle, spike and grains has been associated with Cu-deficiency (Gartrell *et al.*, 1979;

Piening *et al.*, 1985). Piening and MacPherson, (1985) reported that the symptoms of dark pigmentation are due to bacterial infections (*Pseudomonas cichorii*). However, some of the symptoms may not be alleviated with the application of Cu fertilizers. Piening *et al.* (1985) in Alberta demonstrated a reduction of Cu-deficiency symptoms on wheat cv. Park by amending the Cu-deficient soil with $CuSO_4$.

1.3.2 Soil and tissue analysis.

Copper deficiency is often detected by soil tests, plant tissue analysis or controlled yield response experiments. Several soil analysis techniques have been used to predict Cu-deficiency so as to determine the need to apply Cu fertilizer. In soil analysis, DTPA is among the several extractants used in the determination of available Cu (Lindsay and Norvell, 1978). However, this is an empirical method which does not account for the Cu solubilized during extraction. Solubilized Cu is normally what the plant roots absorb, but the Cu level detected in the soil is a separate factor from the genetic potential of a cultivar for Cu uptake and utilization. Nevertheless, plant tissue analysis is still the most precise and accurate diagnostic tool to evaluate the current Cu status of plants (Gartrell *et al.*, 1979; Robson and Reuter, 1981). The Cu levels in plant tissues depend on the plant species, plant organ, growth stage, and the plant environment (Bates, 1971). Walker and Loneragan, (1981) showed that subterranean clover (*T. subterranean* cv. Geraldton) which was supplied with Cu had 2 $\mu g\ g^{-1}$ in old leaves and stems, 3 $\mu g\ g^{-1}$ in the whole tops and 4 $\mu g\ g^{-1}$ in young mature leaves. The ability of specific wheat cultivars to concentrate high amounts of Cu depends on the genotype (Smilde and Henkens, 1967; Nambiar, 1976; Clark, 1983). Copper has been found to be immobile from the leaves at early growth stages, but becomes mobile at the beginning of senescence (Hill *et al.*, 1979; Loneragan *et al.*, 1980). Therefore the use of young leaf blades for determination of Cu accumulated in plants still remains the most sensitive and accurate indicator of Cu status in the wheat plant (Loneragan, 1975; Nambiar, 1976; Gartrell *et al.*, 1979; Brennan, 1980). Limited information is available on effects of Cu on grain quality. A study by Flynn *et al.*, (1987), on effects of Cu-deficiency on grain quality demonstrated that low test weight, loaf volume, flour recovery and sticky dough were typical of grains obtained from the nil Cu treatment. Poor dough quality from Cu-deficient grains has been linked to the reduction of the activities of ascorbate and cytochrome enzymes, which are involved in the synthesis of stored proteins (Walker and Loneragan, 1981). In order to accurately evaluate the status of Cu in wheat plants, the appropriate diagnosis should involve the use of visual Cu-deficiency symptoms, coupled with soil and plant tissue analysis.

1.4.0 Remediation of copper deficiency.

1.4.1 Fertilizer application.

When soils are limiting in Cu, either Cu must be added to correct the deficiency or plants which can tolerate low soil Cu levels may be grown . Several Cu-based compounds have been used to amend Cu-deficient soils. Copper sulphate ($CuSO_4 .5H_2O$), copper oxychloride ($CuCl_2.2CuO.4H_2O$), copper oxide (Cu_2O) and copper chelates (Na_2CuEDTA, Na Cu HEDTA) have been used on different Cu-deficient soils world wide (Reuter, 1975; Glikes, 1981). The effectiveness of these compounds depends largely on crop species, environment and method of application. Foliar Cu applications are effective in correcting Cu-deficiencies in crops (Reith, 1968; Grundon, 1980; Graham and Nambiar, 1981), and they are used for emergency treatments where deficiency is recognized early enough. Although their effectiveness is high, foliar fertilizers have low residual value. As a result, their use should be routine until the accumulated Cu in the soil reaches a sufficient level for crops (Gartrell, 1981). The residual value and efficacy of applied copper fertilizers are important factors because they determine the rate and frequency of application. However, Brennan (1991) observed no residual effects from Cu applied in the previous 24 years, in a wheat crop grown on gravelly grey sandy soil

1.4.2 Breeding and improvement for copper efficiency.

Long term solutions for the improvement of Cu-efficiency are urgently required. Selecting Cu-efficient cultivars and breeding wheat for Cu-efficiency might be a more cost effective alternative than applying copper fertilizers to Cu-deficient soils. However, some criteria need to be fulfilled (Graham, 1984; Sherrard *et al.*, 1986). Any procedure for evaluation of Cu-efficiency must have the potential to allow new genotypes to express differences, and the genotypes being evaluated must have sufficient variability for Cu-efficiency so that improvement through plant breeding can be made. The selectable trait must also have a fairly high heritability.

The transfer of Cu-efficiency genes(s) from chromosome 5RL of rye to wheat has been successfully achieved through triticale (Graham, 1978). Interspecific hybridization between wheat and triticale, and the use of monosomic rye additional lines (Ren *et al.*, 1990) are some of the sources of wheat-rye translocated chromosomes. Several wheat lines with 5RL translocated chromosomes have been bred and utilized in Australia (Graham, 1984; Graham *et al.*, 1988). Nutrient uptake by efficient genotypes depends on root soil interactions. Phytosiderophores (for example, epi-3-hydroxymugenic acid) have been identified in the exudates released from apical root zones of members of the Gramineae family and have been associated with mobilization and uptake of micronutrients, particularly iron (Fe) (Marschner *et al.*, 1987). Phytosiderophores have been found to be released by the roots of Fe-efficient cultivars of oats (*Avena byzantina* C. Koch.), corn (*Zea mais* L.) (Brown *et al.*, 1991), and barley (*H. vulgare*) (Marschner *et al.*, 1986). Marschner *et al.*, (1989) reported that phytosiderophores mobilize not only Fe, but also Zn, Cu and Mn in calcareous soils. This report was further supported by Zhang *et al.*, (1991) who indicated that phytosiderophores have high affinity for micronutrients in the order of $Cu^{2+} > Fe^{3+} > Zn^{2+} > Mn^{2+}$. The 5RL translocated wheat lines developed have shown an increase of Cu uptake, and grain yield by 100% when grown on Cu-deficient soil

(Graham *et al.,* 1988). Consequently, the method of breeding cereals for high Cu uptake using Cu-deficient soil systems seems appropriate.

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CHAPTER 2

RESPONSE TO COPPER OF EIGHT CANADIAN SPRING WHEAT (*T. aestivum* L.) CULTIVARS : I. POLLEN VIABILITY, YIELD AND YIELD COMPONENTS

2.0.0 INTRODUCTION

Copper deficiency affects grain yield directly and indirectly. During the reproductive phase, Cu-deficiency directly causes pollen sterility, a major reduction of pollen number, and dented, small, and starchless pollen in wheat (*T. aestivum*) and barley (*Hordeum vulgare*) (Graham, 1975; Jewell *et al.,* 1988). These findings led to the hypothesis that insufficient carbohydrates in pollen grains cause infertility. Graham *et al.* (1976) suggested that copper deficiency interferes with microsporogenesis during the process of meiosis at the early booting stage in wheat plants. However, the reduction in seed set is due to sterility caused by inviable pollen or failure of stomia to rupture due to decreased lignification of the anther walls in Cu-deficient wheat, oat (*Avena sativa*) and barley (Dell, 1980).

Because of Cu-deficiency in some soils, several approaches to correct Cu-deficiency have been used to avoid significant grain yield losses in wheat. One of the preferred approaches has been the application of copper-based fertilizers. Copper sulphate and copper oxide have been used for commercial wheat production in Australia (Graham *et al.*, 1981; Alloway *et al.*, 1984). Copper oxychloride, an insoluble compound, has been used routinely in commercial wheat farms in Kenya for over two-and-half decades (Pinkerton, 1967). The powdered compound has been used for seed dressing at the rate of 1 kg per 100 kg seed, and this is followed by one foliar application of tank mixture with herbicides at the rate of 1 kg in 200 L H_2O ha^{-1}. The application of copper oxychloride has been noted to provide the multiple effects on the plant by increasing vigour and grain yield. The direct contribution to yield is due to the positive physiological effects of the absorbed Cu in the leaves and in addition, fungicidal properties of copper oxychloride protect the wheat crop from some potential disease infections (Graham, 1984). Various Cu compounds have shown differences in efficacy as soil and foliar copper fertilizers on wheat and barley. For example, copper oxide is ineffective in the year of application but alleviates the deficiencies in the subsequent year while copper sulphate (blue stone) and chelated products are effective in the year of application (Karamanos *et al.*, 1986).

Due to the high costs of copper fertilizers, and their erratic efficacy, their usage has been uneconomical in large scale wheat production, particularly in developing countries where farmers depend on imported fertilizers. Applications from 0.7 to 23 kg Cu ha^{-1} have been proposed as adequate for correcting copper deficiencies in different soils (Reuter, 1975), depending on the environmental conditions, crop species, and form of Cu fertilizers applied. Poor placement in the soil in relation to the plant roots reduces the efficacy and uptake of Cu-fertilizers (Grundon, 1991). The efficiency of Cu uptake increases with the incorporation of fine particles of Cu fertilizer into the soil in such a way that roots have maximum interception (Glikes, 1981). Liquid and fine powder Cu fertilizers have been found to be more effective in supplying Cu^{2+} ions which are absorbed by the plants (Gartrell, 1981).

Weather and weather related variables, such as soil moisture, have an influence on the availability of Cu in the soil for root uptake. Reuter *et al.* (1981) and Grundon (1991) observed an interaction between Cu supplied and soil moisture as they affected growth of subterranean clover and wheat. Their study showed that, although there was a high amount of Cu supply, low soil moisture enhanced Cu-deficiency. Inadequacy of Cu due to low moisture in the soil has been attributed to immobilization of Cu into the soil lattices (Grundon, 1991).

The objective of this field study was to determine the response of eight Canadian spring wheat cultivars to Cu on the basis of grain yield and yield components on an orthic, dark grey, Chernozemic, Cu-deficient soil at Stony Plain, Central Alberta.

2.1.0 MATERIALS AND METHODS.

2.1.1 Site.

A field experiment was conducted for two seasons (1990 and 1991) at Stony Plain, Central Alberta (53° 02' N - 114° 00' W), 50 km west of Edmonton. The soil at the experimental site was an orthic dark grey Chernozemic which originated from deltaic material (Alberta Soil Survey, 1970), and was characterized to be deficient in Cu.

Table 2.0.0 Characteristics of the orthic dark grey Chernozemic soil sampled from the experimental site (Stony Plain) in 1990.

Characteristic	Content
Soil pH ($CaCl_2$)	5.41
Cu	0.48
B	0.45
Mn	71.21
Zn	14.05
Fe	164.60
Organic matter	8.90%

Micronutrient in $\mu g\ g^{-1}$ soil, analysed using DTPA

The farmer's field on which the experiment was conducted was previously sown to a barley crop. Prior to the beginning of the experiment, soil samples from a 0-15 cm furrow slice were collected, bulked and analysed (Table 2.0.0).

2.1.2 Treatments.

The eight Canadian spring wheat cultivars (Katepwa, Roblin, Park, Laura, Conway, Oslo, Columbus, and Biggar) that were used in this study were selected on the basis of their varying agronomic characters. Cultivars Katepwa and Columbus are medium and late (respectively) in maturity, but Roblin and Park are early in maturity. All are awnless. Park has been reported to be sensitive to Cu-deficiency (Piening *et al.*, 1985).

Cultivar Oslo is semi-dwarf, awned, medium in maturity and well adapted to Central Alberta, Biggar is semi-dwarf, awned and late in maturity. Conway and Laura are medium in maturity. In 1990 the land was prepared using a disc plough until a fine tilth suitable for seeding wheat was achieved. In 1991 each plot was tilled and rotovated separately to avoid mixing of the soil from +Cu plots with that in the -Cu plots from the previous season treatments. The 1990 experimental area was demarcated accurately for subsequent placement of the 1991 trial.

A split-plot experimental design with four replicates was used with cultivars sown in the same plots for the two growing seasons. Cultivars were the main-plot factor, and copper (Cu) treatment was the sub-plot factor. Trials were seeded with a disc drill at 17.5-cm row spacing. The plot sizes were 13.7 m long by 1.4 m wide. The seeding rate was 84 kg seed ha^{-1}, using seed of 95% or better germinability. The seed used was dressed with Vitavax to control seed borne and soil diseases. The sub-plots were separated by 0.5 m wide non-planted alleys and 4.8 m between the two blocks. Nitrogen, phosphorus, potassium and sulphate were applied at the rate of 67 kg ha^{-1} (N), 50 kg ha^{-1} (P), 34 kg ha^{-1} (K) and 11 kg ha^{-1} (S). Copper sulphate fertilizer was supplied at the rate of 12.2 kg ha^{-1} in 1990 and as chelate in 1991 on +Cu treatment, while in the control no copper was applied (-Cu). Weeds were controlled by pre-emergence herbicide (triallate) at the rate of 1.5 kg ha^{-1} and post-emergence herbicide (diclofop-methyl) at the rate of 0.78 kg ha^{-1} 28 days after sowing The non-planted alleys were mechanically weeded using a rotovator. The distribution of rainfall and the mean maximum and minimum temperatures for each growing month were recorded (Table 2.1.0). Total growing season precipitations was 349 mm in 1990 and 306 mm in 1991.

2.1.3 Pollen viability.

The purpose of staining pollen grains with iodine-potassium iodate was to identify cultivars which produce sterile pollen grains due to Cu deficiency. Five spikes that had

50% anthers extruded at anthesis were sampled at random from each sub-plot from all four replicates. Spikes were labelled and placed in conical flasks filled with water to avoid wilting during transportation from the experimental site. The following procedure was then used to assay pollen viability. The glumes and palea were clipped, from each spike, which was then left for a few minutes to allow the anthers to extrude and dehisce. Each spike was gently tapped over a slide for the dehisced anthers to shed the pollen grains. The pollen grains were immediately stained with 80% ethyl alcohol (C_2H_5OH) and iodine-potassium iodate (I_2-KI) solution for 90 seconds and covered with plastic slips, using the method of Graham and Pearce (1979). A sample of 250 pollen grains was counted per spike under a light microscope (×400), and non-stained grains were noted. Only pollen grains that were fully stained were considered to be viable. The percent pollen stain was then calculated.

Table 2.1.0 Mean rainfall and temperatures experienced during 1990 and 1991 at the Stony Plain .

		Month			
		May	June	July	August
1990					
Rainfall (mm)		50.6	52.7	159.6	85.6
Mean Temp. °C	Max.	16.6	19.7	21.6	21.7
	Min.	4.9	9.5	11.5	11.3
1991					
Rainfall (mm)		98.7	105.5	24.0	78.0
Mean Temp. °C	Max.	16.7	18.5	22.7	24.4
	Min.	5.3	9.6	11.5	12.6

2.1.4 Yield and yield components.

Most of the sub-plots were harvested at the beginning of September in 1990, but in 1991, harvesting was done at the end of August. From the second half of each sub-plot, ten plants were sampled at random from the inner six rows, for height and spike length measurements. After determining the number of tillers plant^{-1}, the spikes from the main stems and tillers were separated for the determination of the number of florets spike^{-1}, grains spike^{-1}, percent florets spike^{-1}, mean grain weight, and grain yield plant^{-1}. Grain weights were determined by weighing a sample of 100 grains from spikes of the main stems and tillers selected at random. Percent fertile florets spike^{-1} was computed by calculating the ratio of the number of grains spike^{-1} to the number of florets spike^{-1}. The percent Cu-efficiency was calculated by expressing grain yield from -Cu treatment as percent of +Cu treatment of each replicate.

2.1.5 Statistical analyses.

Combined split-plot analyses were used to assess the differences among cultivars and treatments, and cultivar × Cu interaction for grain yield, and yield components. The data for percent stained pollen, fertile florets and percent Cu efficiency for each replicate were transformed using arc sine so as to obtain homogeneous variances. Student *t* -test was used for comparing significant differences between (+Cu) and (-Cu) treatment means of each cultivar for grain yield and some yield components.

2.2.0 RESULTS.

2.2.1 Yield and yield components.

2.2.1.1 Pollen viability, grain yield, and yield components.

The light microscope observations showed that the sizes of pollen grains varied with cultivar. However, pollen grains from Cu-deficient treatments showed dented conformations. Two categories of stained pollen were observed, dented and non-stained pollen which indicated sterility, and some which were partially stained. The results (Table 2.2.0) showed significant ($P \leq 0.01$) main effects due to cultivar and Cu for grain yield and

Table 2.2.0 Mean squares for grain yield and yield components of eight Canadian spring wheat cultivars (*T. aestivum* L.).

Source	df	% Pollen stain	% fertile florets spike^{-1}		Number of grains spike^{-1}		Grain weight (mg)		Grain yield g plant^{-1}.
			Main stem	Tillers	Main stem	Tillers	Main stem	Tillers	
Rep	3	95.1	210.6	289.6	50.6	171.8	9.7	1.5	0.4
Cultivar	7	184.3**	358.9**	510.0**	902.0**	509.5**	45.6**	71.8**	25.3**
Cultivar × Rep (E$_a$)	21	22.8	37.8	41.2	35.5	25.3	10.4	9.3	0.6
cv (%)		7.1	11.2	12.5	15.2	23.8	9.3	9.3	18.3
Cu	1	735.8**	669.2**	1393.2**	697.8**	819.1**	44.3**	1.4	19.4**
Rep × Cu	3	11.3	50.7	34.6	33.8	35.5	26.0	15.6	0.2
Cultivar × Cu	7	43.4	45.0	61.8	46.1	43.4	9.9*	6.0	1.9
Rep × Cu × cultivar (E$_b$)	21	28.1	37.8	31.6	35.1	31.0	3.5	6.2	1.5
cv (%)		7.8	11.2	11.0	17.8	22.3	5.3	7.7	27.3
Year	1	1337.3**	474.5**	1017.3**	83.8	424.1**	236.3**	75.8**	9.7**
Year × Cultivar	7	46.4	87.2**	92.3*	40.1	29.9	45.5**	48.0	3.3*
Year × Cu	1	84.7	207.0**	187.7*	301.5**	76.0	22.2	24.2	19.1**
Year × Cultivar × Cu	7	15.8	67.4*	50.5	61.7*	28.5	8.3	8.0	1.4
Error	48	27.8	27.3	38.2	24.1	23.0	8.6	8.5	1.2
cv (%)		7.8	9.6	12.1	14.8	19.2	8.4	8.9	24.6

*, ** significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.
cv coefficient of variation
E Error

Table 2.2.1 Mean squares for the number of florets, spike length, height and the number of tillers plant^{-1} of eight Canadian spring wheat cultivars (*T. aestivum* L.).

Source	df	Number of florets spike^{-1}		Spike length (cm)		Height (cm)	Tillers plant^{-1}
		Main stem	Tillers	Main stem	Tillers		
Rep	3	63.3	8.4	0.3	0.3	241.3	1.1
Cultivar	7	819.0**	436.6**	7.8**	9.7**	1813.9**	10.2**
Cultivar × Rep (E_a)	21	28.8	10.1	0.1	0.2	41.7	0.9
cv (%)		10.7	7.7	3.5	5.5	6.7	16.8
Cu	1	91.1	76.8	0.4	0.7*	587.8**	0.2
Rep × Cu	3	76.4	9.2	0.1	0.1	91.5	0.5
Cultivar × Cu	7	22.1	15.7	0.1	0.1	18.2	1.3
Rep × Cu × cultivar (E_b)	21	30.3	23.3	0.1	0.1	42.4	0.8
cv (%)		10.9	11.7	4.6	5.0	6.8	16.0
Year	1	205.0**	1.3	1.3	0.5	44.7	23.2**
Year × Cultivar	7	49.8*	37.0	1.1**	0.3	37.2	3.6
Year × Cu	1	111.4*	0.2	0.1	0.1	374.0**	0.1
Year × Cultivar × Cu	7	18.1	6.9	0.1	0.2	16.2	0.6
Error	48	19.1	17.1	0.1	0.2	32.9	1.0
cv (%)		9.4	10.5	3.8	5.7	5.9	17.6

*, ** significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.
cv coefficient of variation.
E Error.

various yield components. Significant ($P \leq 0.05$) cultivar × Cu interaction was observed for grain weight from spikes of main stems.

Growing season effects were significant ($P \leq 0.01$) for grain yield and yield components. Significant ($P \leq 0.05$) year × cultivar interaction for floret fertility, grain weight and grain yield indicated differential performance of the test cultivars in different seasons. Significant ($P \leq 0.05$) year × Cu interaction for floret fertility, number of grains spike^{-1}, and grain yield suggested that the effects of applied soil Cu on the cultivars tested was dependent on the prevailing environment. For floret fertility and the number of grains spike^{-1} of main stem, a significant ($P \leq 0.05$) interaction for the year × cultivar × Cu effects was observed. This interaction illustrated that the test cultivars responded differently to Cu supply in different growing seasons.

Cultivar effects were significant ($P \leq 0.01$) for number of florets spike^{-1}, spike length, height and number of tillers plant^{-1} (Table 2.2.1). Significant ($P \leq 0.05$) effects due to Cu were observed on the length of spikes, on the tillers, and on height. Year effects were significant ($P \leq 0.01$) for the number of florets spike^{-1} of the main stems, and the number of tillers plant^{-1}. Significant ($P \leq 0.05$) year × cultivar interactions for the number of florets spike^{-1} and the length of spikes from the main stems were observed. The year × Cu interaction was significant ($P \leq 0.05$) for the number of florets spike^{-1} of main stems and for height.

2.2.1.2 Effects of copper on pollen viability, grain yield, and yield components.

Results of pollen viability and floret fertility (Table 2.3.0) indicated significant ($P \leq 0.05$) percent viable pollen response to Cu for all cultivars except Laura and Biggar. Biggar showed the highest mean pollen viability while Roblin had the lowest in the -Cu treatment. The highest mean percent viable pollen was observed on cv. Katepwa in the +Cu treatment. Copper treatment significantly ($P \leq 0.05$) improved the floret fertility of Roblin, Laura, Conway and Oslo. The highest response to Cu for floret fertility was observed on cv. Laura. No significant responses to Cu level were observed for the remaining cultivars.

Table 2.3.0. Effects of copper on pollen viability and floret fertility of eight Canadian spring wheat cultivars

	% Pollen stain		% fertile floret spike^{-1}			
			Main stems		Tillers	
Cultivar	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu
Katepwa	84.4	92.3**	71.0	76.4	67.0	75.3
Roblin	71.6	85.6**	60.0	67.4*	51.1	62.3*
Park	72.9	80.9*	58.2	65.5	46.6	61.2
Laura	77.8	83.5	53.6	70.1*	43.9	60.9*
Conway	83.5	89.0**	63.4	71.5*	58.1	70.0*
Oslo	81.2	88.2*	50.7	65.4*	38.6	57.5*
Columbus	84.5	86.6*	59.3	60.9	59.2	60.8
Biggar	87.4	88.0	78.0	80.1	73.1	74.8

*, ** - significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.

The response to Cu application of mean grain weight, the number of grains spike^{-1}, and grain yield (Table 2.3.1) varied by cultivar. Significant ($P \leq 0.05$) increases in grain weight of the main stems were observed for cvs. Katepwa, Laura, Conway, and Oslo. Cultivar Columbus showed the highest mean grain weight on both -Cu and +Cu treatments, but no significant increase in grain weight due to Cu application was observed on grains from the tillers. Cultivar Conway increased grain weight on the main stem by 10% when supplied with Cu.

Table 2.3.1 Effects of copper on the number of grains spike^{-1} and grain yield, and Cu-efficiency for grain yield of eight Canadian spring wheat cultivars.

	Mean grain weight (mg)				Number of grains spike^{-1}				Grain yield g plant^{-1}		% Efficiency for grain yield
	Main stems		Tillers		Main stems		Tillers				
Cultivar	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	
Katepwa	35.3	37.5*	33.9	35.3	31.3	32.0	26.9	31.3	5.93	6.85	91.3 ab
Roblin	35.5	35.2	34.8	32.9	27.5	31.8**	18.9	25.1**	3.18	3.98**	79.6 ab
Park	31.3	33.2	30.0	30.8	26.7	31.4*	17.0	24.0*	2.93	3.70	86.0 ab
Laura	32.2	34.2*	30.4	32.1	29.9	37.2	18.8	24.7	2.87	3.87	82.5 ab
Conway	32.7	36.1*	32.0	32.8	28.1	30.4	21.1	25.3*	4.31	4.79	94.7 a
Oslo	34.1	36.0*	32.2	31.0	27.0	38.0**	17.7	28.6**	2.75	4.77**	57.6 b
Columbus	38.1	36.9	35.8	35.9	25.8	27.7	21.7	23.2	3.83	4.63	84.0 ab
Biggar	34.3	34.0	29.8	29.8	50.5	50.9	37.1	37.6	6.61	6.06	108.4 a
											Lsd 0.05 34.3
											Cultivar† *
											Year **
											Cultivar × Year *

*, ** - significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.
For percent Cu-efficiency, means with the same letters are not significantly different
† Detailed analysis of variance is explained in Appendix 2.0.0

Significant ($P \leq 0.05$) response to Cu for number of grains spike^{-1} were observed on the main stems and tillers of cvs. Roblin, Park and Oslo. Cultivar Conway showed significant ($P \leq 0.05$) response to Cu for the number of grains spike^{-1} from tillers. Cultivar Biggar showed the highest number of grains produced on spikes from both the main stems, and tillers. The lowest number of grains spike^{-1} were observed on cv. Columbus.

Significant ($P \leq 0.05$) effects due to cultivar, year, cultivar × year for Cu-efficiency for grain yield were observed. Cultivar Biggar showed the highest grain yield in the -Cu treatment and the highest percent Cu-efficiency (Table 2.3.1). Although cv. Conway showed high Cu efficiency, its grain yield was comparatively lower than cv. Biggar. The lowest grain yields were observed on cv. Oslo with the lowest percent Cu-efficiency among the tested cultivars. Significant ($P \leq 0.05$) Cu effects were observed on cvs. Roblin, and Oslo for grain yield.

2.3.0 DISCUSSION

Pollen viability

One of the major effects of Cu-deficiency in wheat is to cause pollen grain sterility. In this study, test cultivars differed significantly in pollen viability, and supply of Cu improved the percent viability of the pollen grains. Fewer dented and starchless pollen grains from the +Cu treatment were observed compared to the -Cu treatment and this suggested that Cu is essential for full pollen grain development and viability. Similar findings about pollen grains have been reported on wheat deficient in Cu (Graham, 1975; Graham and Pearce, 1979). The use of starch dye to estimate pollen viability has been criticized (Jeffers, 1977) and the use of this technique in this study meant that verification of the viability of partly stained or fully stained pollen grains was not possible. However, the high percent pollen grain stain of the tested cultivars did verify that starch was present. Based on the results of the histochemical reactions, the number of stained pollen grains were sufficient to initiate fertilization on pistils without causing drastic grain set reduction. However, low numbers of grains spike^{-1} were observed on cvs. Roblin, Park and Oslo, particularly on tillers. Therefore, the limited amount of starch in pollen grains was not the sole cause of sterility. Graham (1976) suggested that Cu-deficiency interferes with the microsporogenesis process at the early booting stage, while Jewell, *et al.* (1988) linked the effects of Cu-deficiency on barley to structural defects during the development of anthers and pollen grains. This study showed that sufficient pollen grains for fertilization were produced from dehisced anthers. Therefore, the results did not conform with the findings of Dell (1981), who suggested that Cu-deficiency prevents the anthers of wheat, oats and barley from dehiscing, through a reduced thickening of the endothecial layer of the anthers. Consequently, the staining technique used would be suitable for predicting pollen viability in severe Cu-deficiency situations.

Yield and yield components.

The tested cultivars showed differences in yield potential on Cu-deficient soil (Table 2.3.0). Similar genotypic differences in wheat cultivars for Cu nutrition have been reported (Smilde and Henkens, 1967; Nambiar, 1976 and Graham 1984; Graham *et al.*, 1987). Significant Cu effects indicated the need to supply Cu to wheat plants at Stony Plain. Nevertheless, significant ($P \leq 0.01$) year effects and interactions with year effects, for yield and yield components also imply that in the same site, seasonal variation can also have significant effects on Cu nutrition and the productivity of wheat. Variation between the seasons (Appendix 2.1.0) could be accounted for by the differences in rainfall which was higher and well distributed in the first year compared to the second year of the trial.

Due to variations in seasons, the test cultivars performed differently in the two seasons as indicated by the significant ($P \leq 0.05$) year × cultivar interactions for floret fertility, mean grain weight from the main stems, and grain yield. The effects of Cu varied between years for floret fertility, number of grains produced spike^{-1} and grain yield. Again, this could be a result of variation in the rainfall and soil moisture regimes. It has been reported that soil moisture deficit enhances Cu-deficiency in wheat by immobilization of Cu into soil lattices (Graham and Nambiar, 1981). Significant ($P \leq 0.05$) year × cultivar × Cu effects for floret fertility and for number of grains spike^{-1} indicated that wheat cultivars responded differently to Cu supply under the different environments of the two seasons.

Effects of copper on pollen viability, grain yield and yield components.

Six of the eight cultivars tested responded to Cu with increase in percent pollen viability. Cultivars Katepwa, Park, Columbus and Biggar did not show significant response to Cu for floret fertility. However, the magnitude of response varied among cultivars. The quotient that was used to determine floret fertility in the experiment was a function of the number of grains spike^{-1} and the number of florets spike^{-1}. The positive effects of Cu on pollen viability accounted for the increase of percent floret fertility of cvs. Roblin, Conway, Oslo. Therefore, it was evident that the supply of Cu to the three cultivars increased pollen viability, and percent floret fertility, which further accounted for an increase in the number of grains spike^{-1} (Table 2.3.1). Cultivar Park responded significantly ($P \leq 0.05$) to Cu supply for number of grains spike^{-1}. The results showed that the influence of Cu on the number of grains spike^{-1} depended on the cultivar, and by culm. The effects of Cu on the number of grains spike^{-1} were significant on both the main stems and tillers of cvs. Roblin, Park and Oslo, while on cv. Conway, the influence was only on the production of grains on tillers.

The weight of the grain indicated the degree of plumpness of the grains, an important agronomic factor. Cultivars Katepwa, Laura Conway and Oslo showed a significant increase in grain weight (5.6 - 10%) of grains from the main stems due to Cu application. No significant increase in grain weight was observed on grains from tillers. Copper is involved in carbohydrate mobilization and remobilization (Brown *et al.*, 1977).

Consequently, Katepwa, Laura, Conway and Oslo appear to have a high demand for copper.

The addition of Cu increased significantly the grain yield of cvs. Roblin and Oslo but grain yield increase was not significant for Katepwa, Park, Laura, Conway, Columbus and Biggar, (Table 2.3.1). Among the eight cultivars, Biggar showed significantly higher grain yield than other cultivars with the highest percent Cu-efficiency (108.4%), and positively responded to Cu with a 9% yield increase. By contrast cv. Oslo showed the lowest yield, with the lowest Cu-efficiency (57.6%) and positively responded to Cu application with a 73% grain yield increase. Although cv. Conway showed high efficiency, it was vulnerable to seasonal variations, and performed poorly under lower rainfall regime of 1990. Cultivars Roblin and Park showed positive responses of 25-26% to Cu, and Cu-efficiencies of 79.6% and 86.0% respectively. These two cultivars are early maturing with similar growth rates. Chapin (1987) suggested that plants with traits for a slow developmental rate have a low demand for nutrients, and this could be the case for cv. Biggar. However, this hypothesis do not hold true for cv. Oslo which is medium in maturity, but was sensitive to Cu-deficiency. Also, cultivars Roblin and Park, which are early maturing cultivars had a high demand for Cu. Significant cultivar × year interaction for percent Cu-efficiency indicated that Cu efficiency of the tested cultivars varied with the year.

2.4.0 CONCLUSION

This field study demonstrated that the effects of Cu-deficiency on grain yield, yield components pollen viability and other traits varied with the cultivars. Cultivars Oslo, Roblin, Laura and Park were sensitive to Cu deficiency, while cv. Biggar was found to be the most tolerant of Cu deficiency. The sensitivity of the remaining cultivars depended on the environment and differences between them were more pronounced in the year of lower rainfall.

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CHAPTER 3

RESPONSE TO COPPER OF EIGHT CANADIAN SPRING WHEAT (*T. aestivum* L.) CULTIVARS : II. COPPER CONTENT IN THE LEAVES AND GRAINS.

3.0.0 INTRODUCTION.

Plant tissue analysis is a useful diagnostic tool for evaluating the current nutritional status of plants. Prior knowledge of the mobility of Cu within the plants is essential in deciding which plant tissue to sample. Copper is immobile at early stages of plant growth, but becomes mobile at the beginning of senescence in wheat (Hill *et al.*, 1979; Loneragan *et al.*, 1980). Walker and Loneragan (1981), reported that subterranean clover (*T. subterranean* cv. Geraldton) supplied with Cu had 2 $\mu g\ g^{-1}$ in the older leaves and stems, 3 $\mu g\ g^{-1}$ in the whole tops, and 4 $\mu g\ g^{-1}$ in young leaves. Reuter *et al.* (1981b) observed critical Cu concentration of 3 $\mu g\ g^{-1}$ on the young leaf blades of subterranean clover (*T. subterranean* cv. Seaton Park). Copper concentration in plants depends on the species, genotypes, prevailing environmental conditions, plant organ sampled and age of plant at sampling time (Graham and Nambiar, 1981). Due to a wider range of Cu concentrations in plant organs and between Cu-deficient and sufficient plants, the youngest fully emerged leaves (YFEL) have been the most appropriate organs to use for evaluating Cu status of the whole plant. Therefore, the use of young leaf blades for detection of Cu in plants still remains the most sensitive and accurate indicator of the Cu status of wheat (Loneragan, 1975; Nambiar, 1976b; Gartrell *et al.*, 1979b; Brennan, 1980) and for subterranean clover (*T. subterranean* cv. Seaton Park) (Reuter *et al.*, 1981b).

In wheat grown under greenhouse conditions, the critical Cu concentration at mid-tillering of the youngest fully emerged leaves has been reported to be 1 $\mu g\ g^{-1}$ (Nambiar, 1976b). At the stage between seedling to start of senescence of the YFEL the corresponding value is 2.5 $\mu g\ g^{-1}$ under field conditions (Gartrell *et al.*, 1979a). Copper concentrations are relatively high in young growing wheat seedlings and decrease gradually as the plant matures (Gladstones *et al.*, 1975; Loneragan, 1975). Analysis of Cu concentration in YFEL can help in the prediction of Cu status in the leaves at all stages of plant growth. The following classes of Cu concentrations in wheat for Australian conditions have been reported by Graham and Nambiar (1981): <1.3 ppm indicates moderate to severe deficiency, 1.3-1.6 ppm mild deficiency, 1.6-2.0 ppm marginal deficiency, and > 2 ppm healthy plants. Copper concentration in the grains from Cu-deficient wheat plants ranged between 0.88 and 1.58 ppm (King and Alston, 1975). Under field conditions the critical Cu concentrations in the grains has been reported to be 2.5 ppm (Nambiar, 1976b). Although Cu concentration in grains increase in small quantities with an increase of Cu supply, the Cu content in young leaves is a value of high precision for evaluating the sufficiency of Cu supply (Robson and Reuter, 1981).

Wheat cultivars vary in their accumulation and use of Cu. The ability of specific wheat cultivars to grow and produce on Cu-deficient soils, or the way in which a cultivar is capable of concentrating high amounts of Cu in different plant organs depends largely on genotype (Smilde and Henkens, 1967; Nambiar, 1976a; Graham *et al.*, 1988). Differences among cultivars in the uptake of Cu to the developing organs plays a significant role in the Cu-efficiency (Graham, 1978; Graham *et al.*, 1981). Copper deficiency depresses Cu concentration of the shoot tips, and young and mature leaves of subterranean clover (*T. subterranean*) by 35-65%, depresses activities of ascorbate and cytochrome oxidase enzymes in young leaves by 70-95%, and reduces nitrogen in the young leaves by 20% (Walker and Loneragan, 1981). In cereals, Cu-deficiency produces several symptoms which are characteristic and similar over a wide range of environments. However, significant grain yield losses of up to 20% may still occur without visual symptoms (Graham and Nambiar, 1981).

The specific objectives of this study were :-

i) To detect the critical Cu range in the YFEL and in grains from the main stems and tillers of eight wheat cultivars,

ii) To determine the Cu requirements of the eight test cultivars.

3.2.0 MATERIALS AND METHODS

Details of the experimental site and cultural methods were described previously in section 2.1.0. From the same experimental design and treatment, YFEL of the eight test cultivars were sampled (excluding leaf sheath) from one half of each sub-plot. Youngest fully emerged leaves (YFEL) were sampled to provide tissues of the same physiological age from the eight test cultivars grown on Cu-deficient soil (-Cu) and those on soil supplied with Cu (+Cu).

The youngest fully emerged leaves were sampled 21, 28, 35, 42 and 51 days after sowing (DAS) when plants were at the following Zadoks growth stages (Zadok *et al.*, 1974): 12 (two leaves with one shoot), 22 (leaf sheath lengthened, with main shoot and three tillers for 1990; 23 leaf sheath lengthened, with main shoot and three tillers for 1991), 24 (main shoot with four tillers), 25 (main shoot and five tillers) and 39 (flag leaf ligule just visible). The sampled leaves were decontaminated, a procedure that involved rinsing samples three times in distilled water and drying in a forced air oven at 65 ^{o}C for seven days. The dried samples were chopped into small pieces, mixed thoroughly and stored in paper envelopes to await ashing. Samples of whole grains from the main stems and tillers from which yield was determined were subjected to the same procedure as the leaf samples. For the analysis of Cu concentration in the tissue, a method was adopted from an experiment conducted by Taylor (1989). About 0.5 g of dried sample was ashed at 500 ^{o}C for 48 hours, dissolved in 0.4 mL concentrated nitric acid (HNO_3), oxidized with 0.4 mL 50% hydrogen peroxide (H_2O_2) and diluted to 10 mL volume. For 1991 samples, 5 mL of diluted sample was concentrated to dryness in a Speed Vac high speed concentrator SC100 and dissolved in 2 mL of deionized water. Copper concentration was determined by atomic absorption spectrophotometry on a Perkin - Elmer model 3030A spectrophotometer using air-acetylene flame conditions.

Statistical analyses.

Split-plot analyses of variance for Cu concentration in the YFEL were used to assess the effects of cultivars, Cu level, year, growth stage and any interaction. The grains from main stems and tillers were also analysed. In order to determine the relationship between Cu in the leaf blades at different growth stages and yield, and yield components, Cu concentration values in the YFEL at five growth stages were correlated to grain yield, number of the grains spike^{-1} and percent fertile florets spike^{-1}, main stems and tillers.

3.3.0 RESULTS

3.3.1 Symptoms.

The symptoms of Cu-deficiency noted in the field trials differed with the cultivars, stage of growth and season (year). In 1990, symptoms of deficiency included rolling and wilting of the young leaves before emerging fully, twisting and terminal dieback of leaves on cvs. Park and Katepwa grown on -Cu treatment at Zadoks growth stage 22 (28 DAS). At Zadok growth stage 24 (35 DAS), the deficiency symptoms were prominent. These consisted mainly of rolling and dieback of emerging leaves forming 'rat tail' on Park, Roblin and Katepwa and longitudinal splitting of the midrib on the young emerging and young fully opened leaves of Roblin, Katepwa, Park and Oslo. The leaves of sensitive cultivars that were fully extended had necrosis along the edge of lamina and this was observed on cvs. Roblin (Figure 3.0.0), Park, Laura, Katepwa, Conway and Oslo. No deficiency symptoms were noted on cvs. Columbus and Biggar the two late cultivars. The intensity of Cu-deficiency symptoms decreased as the plants developed for cvs. Katepwa, Roblin and Park. The symptoms of the deficiency were also observed on cvs. Park, Katepwa and Roblin on both +Cu and -Cu treatments. Spikes of Oslo, Park and Roblin showed a range of poor grain development and lack of developed grains. The effects of Cu-deficiency were observed on spikes from the tillers of cvs. Oslo and Park.

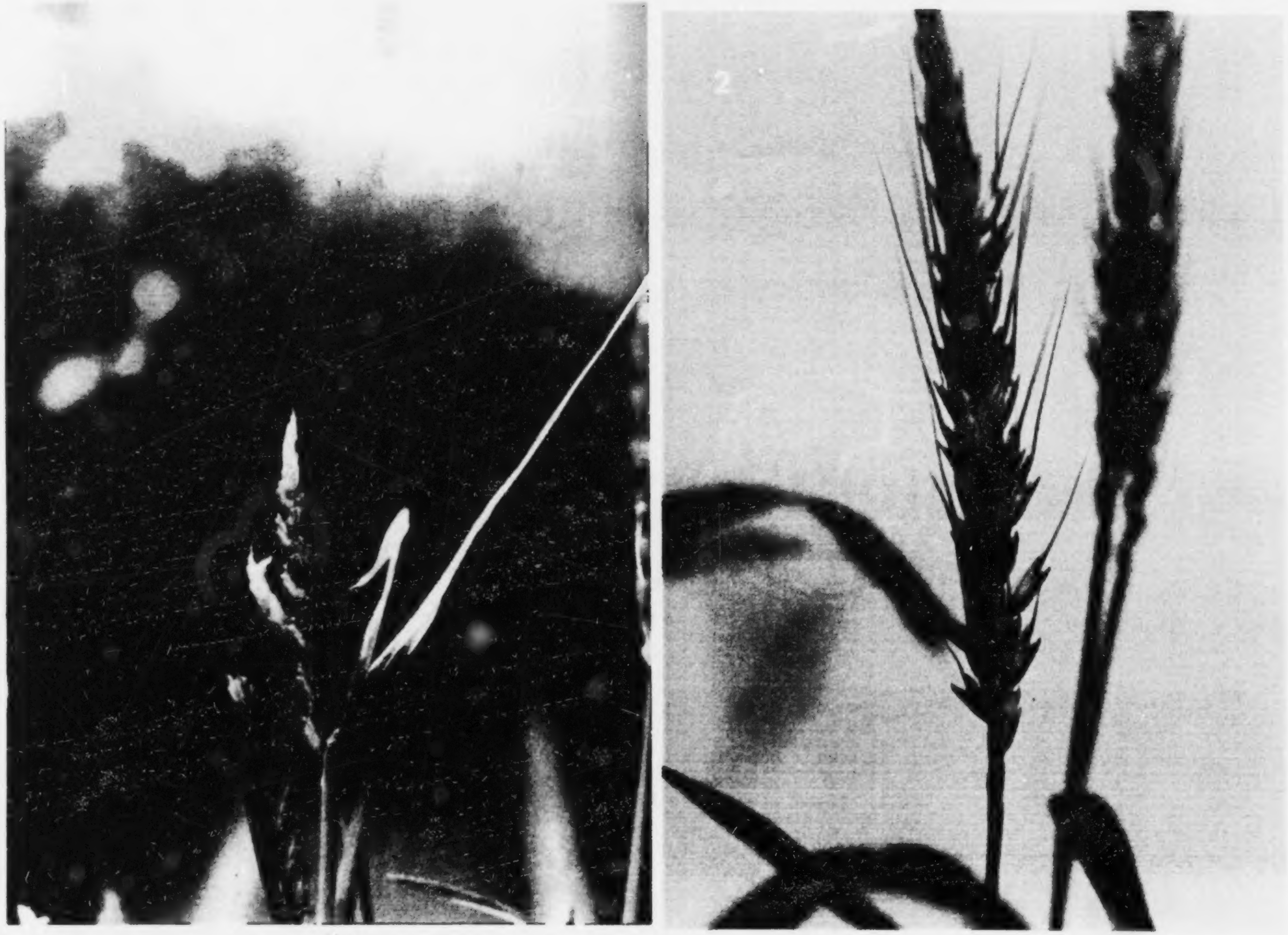


Figure 3.0.0 (1) Leaf and spike dieback on cv. Roblin and (2) floret sterility on cv. Oslo grown on orthic dark grey Chernozemic Cu-deficient soil.

In 1991, Cu-deficiency symptoms appeared at Zadoks growth stage 25 (35 DAS). Symptoms were similar to those noticed in the previous year's experiment, and were first noticed on cvs. Park, Oslo and Roblin from -Cu treatments. However, the symptoms were more conspicuous at Zadoks growth stage 39 (51 DAS) with necrosis of the leaves appearing on cv. Biggar. Unlike in the previous season, the expression of symptoms increased as the plants matured. At maturity in both years, stem and head melanosis was observed on cvs. Park, Katepwa, Roblin and Oslo. On cvs. Park, Katepwa and Roblin, senescence and maturity was also delayed in the -Cu treatment.

3.3.2 Analysis of variance over time for copper concentration in the youngest fully emerged leaves.

The results (Table 3.0.0) showed significant ($P \leq 0.01$) effects due to cultivar, copper treatment, year and growth stages (GS) for Cu level of the YFEL. No significant interactions were observed for cultivar × Cu, or for year × Cu × cultivar. However, significant ($P \leq 0.01$) interactions were observed for GS × cultivar, GS × Cu, GS × year, and ($P \leq 0.05$) GS × year × Cu × cultivar.

3.3.3 Copper concentrations in the youngest fully emerged leaves.

In all the test cultivars, Cu concentrations in the leaves were higher at early plant growth stages and decreased with the maturity of the plants (Figures 3.1.0 and 3.1.1). These results agree with the report of Gladstones *et al.* (1975), and Loneragan (1975). However, the cultivars translocated different amounts of copper to the leaves in different seasons. Higher levels of Cu were detected in the leaves of plants from +Cu treatment than in the -Cu treatment for the cultivars.

In 1990, Cu-deficiency symptoms observed on cvs. Katepwa, Park and Roblin occurred when concentrations of Cu ranged between 4.6 and 5.7 $\mu g\ g^{-1}$ at Zadoks

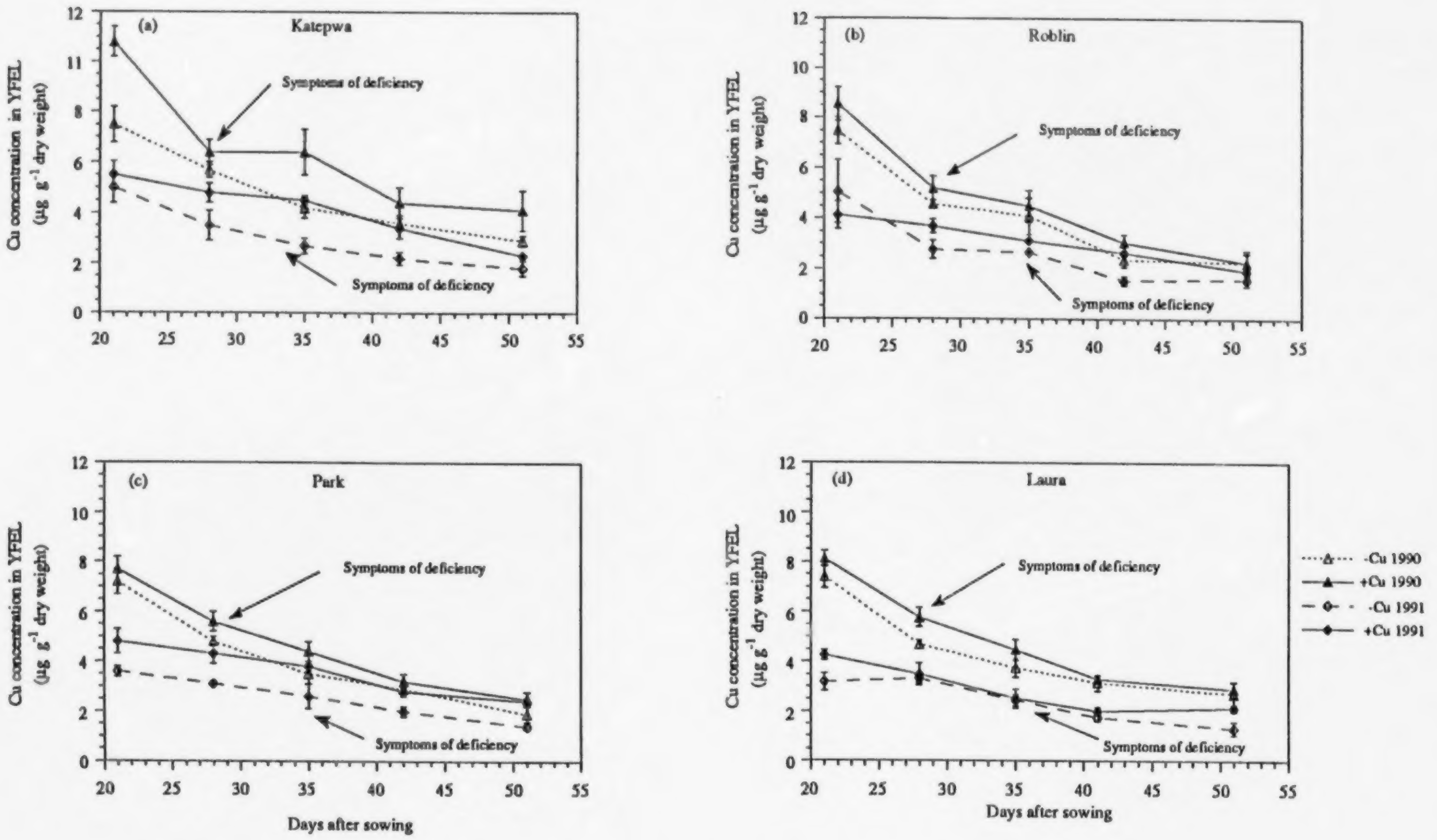


Figure 3.1.0. The relationship between Cu concentration (μg g^{-1} dry weight) in the youngest fully emerged leaves (YFEL) from -Cu and +Cu treatments and time (Days after sowing) of Canadian spring wheat cvs.(a) Katepwa, (b) Roblin, (c) Park and (d) Laura grown at Stony Plain, Alberta.

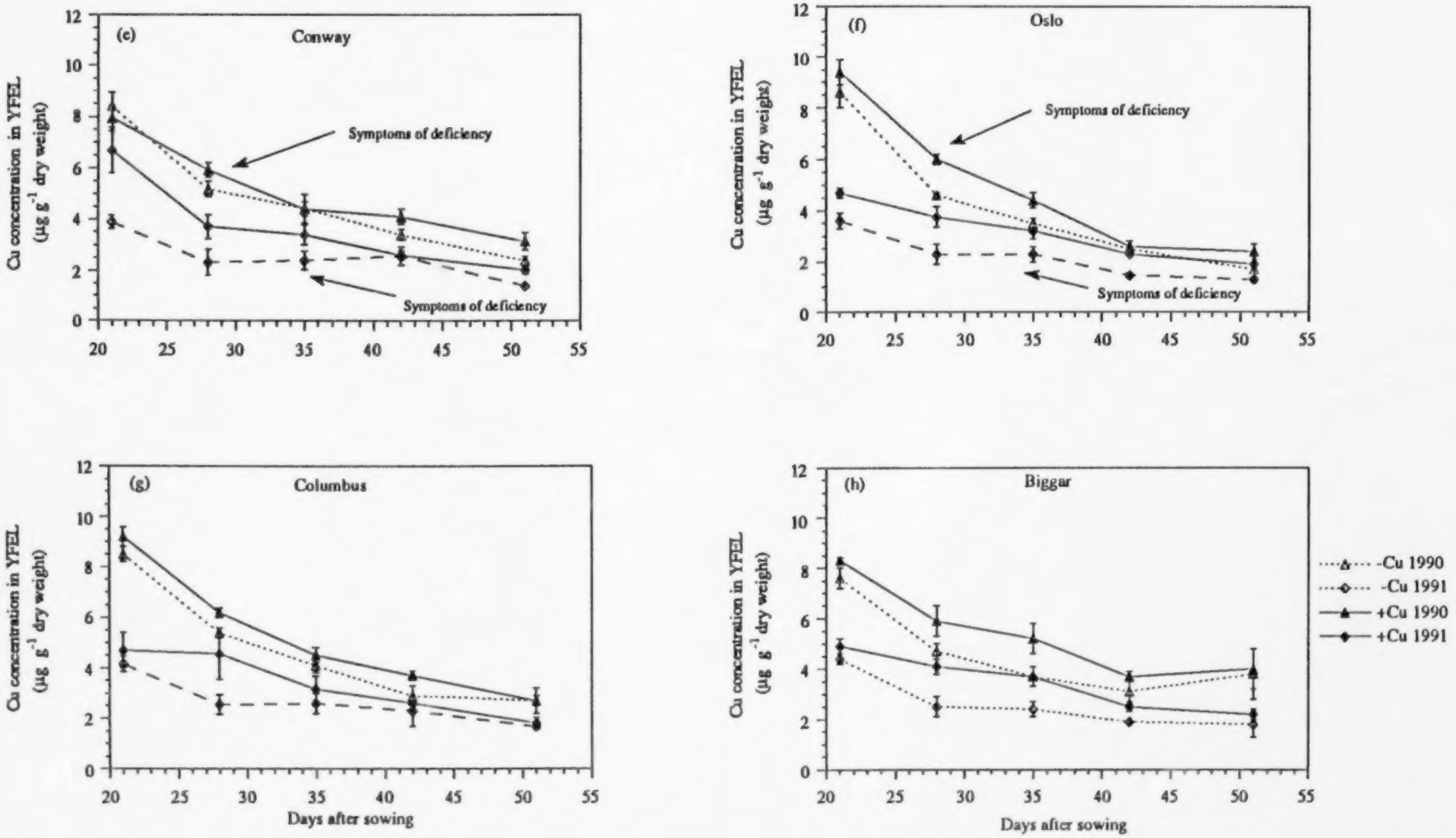


Figure 3.1.1. The relationship between Cu concentration ($\mu g\ g^{-1}$ dry weight) in the youngest fully emerged leaves (YFEL) from -Cu and +Cu treatments and time (Days after sowing) of Canadian spring wheat cvs.(e) Conway, (f) Oslo, (g) Columbus and (h) Biggar grown at Stony Plain, Alberta.

growth stage 22 (28 DAS). At the same stage, the levels of Cu in the YFEL of these three cultivars, from +Cu treatment was between 5.2 and 6.4 $\mu g\ g^{-1}$. However, in the second growing season, the Cu concentration was between 2.8 and 3.5 $\mu g\ g^{-1}$ in the YFEL from the -Cu treatment, and between 3.7 and 4.8 $\mu g\ g^{-1}$ in the +Cu treatment at Zadoks growth stage 22.

Table 3.0.0. Analysis of variance over time for copper concentration in the YFEL of wheat (*T. aestivum*).

Source	df	Mean Squares
Rep	3	5.915
Cultivar	7	8.368**
Cultivar × Rep (Error$_a$)	21	1.519
Cu	1	97.500**
Cu × cultivar	7	1.528
Cu × Rep	3	1.007
Cu × cultivar × Rep (Error$_b$)	21	0.780
Year	1	522.367**
Year × cultivar	7	1.329
Year × Cu	1	0.564
Year × Cu × cultivar	7	0.903
Year × Cu × cultivar × Rep (Error$_c$)	48	1.731
GS	4	331.372**
GS × cultivar	28	0.912**
GS × Cu	4	1.759**
GS × year	4	44.893**
GS × year × Cu	4	0.228
GS × year × cultivar	28	0.697
GS × year × Cu × cultivar	56	0.703*
Error (cv 17.0%)	384	0.475

*, ** = significant ($p \le 0.05$), ($P \le 0.01$), respectively.
GS = growth stage at sampling.

Cultivars Laura, Conway, Oslo, Columbus and Biggar which did not show deficiency symptoms at 28 DAS had Cu concentration of 4.6 and 5.4 μg g^{-1}, in 1990 while in the 1991 season, a range of 2.3-3.1 μg g^{-1} was detected in the YFEL from -Cu treatment (Appendix 3.0.0). These cultivars, when supplied with Cu, showed Cu concentration between 5.8 and 6.3 μg g^{-1} in the first year of the trial and between 3.5 and 4.6 μg g^{-1} in the second year of the trial. Cultivar Biggar which yielded higher in both growing seasons, showed Cu concentrations of 4.7 ± 0.3 μg g^{-1} in 1990, and 2.5 ± 0.4 μg g^{-1} in the second year at Zadoks growth stage 22 (28 DAS).

The flag leaf is an important organ for most of the cereal crops. Cu concentrations in the flag leaves of the test cultivars ranged from 1.7-3.8 μg g^{-1} in the leaf samples from -Cu treatment, and 2.3 - 4.1 μg g^{-1} from +Cu treatment at Zadoks growth stage 39 (51 DAS), in 1990. In 1991 the range of Cu in the leaf blades was 1.3-1.8 μg g^{-1} in wheat plants grown on Cu deficient soil (-Cu), and 1.8-2.4 μg g^{-1} in plants grown in soil ameliorated with copper sulphate (+Cu) in the field. The test cultivars showed low Cu levels in the flag leaves and the differences in Cu content in the flag leaves from +Cu and -Cu treatment were not significant for most of the cultivars. Copper in the flag leaf blades (YFEL) were generally higher in 1990 than 1991. The average Cu concentration in the flag leaf for two years ranged between 1.5 and 1.7 for Oslo and Park, and 2.2 and 2.8 μg g^{-1} dry weight for cvs. Biggar, Katepwa and Columbus from -Cu treatment. For copper treated cultivars, the range was between 2.2 and 2.5 μg g^{-1} for cvs. Oslo and Park, and 2.2 and 3.2 μg g^{-1} for cvs. Biggar, Katepwa and Columbus. Cultivars Biggar and Katepwa accumulated more Cu in the flag leaves in the first season than in the second season.

The grains from tillers showed lower Cu levels than those from spikes of the main stems of cultivars (Table 3.1.0 and Table 3.1.1). In the Cu-deficient soil cv. Park showed the lowest level of Cu in the grains from tillers, while cv. Biggar had higher Cu levels in those kernels in 1991 than in 1990 season. Cultivar Katepwa which showed high

Table 3.1.0. Concentrations of copper (µg g^{-1} dry weight) in grains from spikes of main stems of eightt Canadian spring wheat cultivars.

Treatment		Katepwa	Roblin	Park	Laura	Conway	Oslo	Columbus	Biggar
-Cu	1990	2.2 ± 0.6	2.2 ± 0.6	1.6 ± 0.3	2.3 ± 0.8	2.8 ± 0.4	2.2 ± 0.3	1.8 ± 0.3	1.8 ± 0.5
	1991	1.7 ± 0.2	2.1 ± 0.4	1.6 ± 0.3	1.7 ± 0.6	1.4 ± 0.3	2.7 + 0.8	1.7 ± 0.1	2.0 ± 0.2
	Mean	1.90	2.15	1.60	2.0	2.10	2.45	1.75	1.9
+ Cu	1990	5.9 ± 1.3	2.9 ± 1.2	2.9 ± 0.8	3.3 ± 0.8	2.6 ± 0.7	2.4 ± 0.8	2.0 ± 0.8	2.9 ± 0.7
	1991	2.8 ± 0.3	2.6 ± 0.4	1.9 ± 0.2	2.3 ± 0.8	2.6 ± 0.3	2.1 ± 0.3	2.5 ± 0.3	2.9 ± 0.4
	Mean	4.35	2.75	2.40	2.80	2.60	2.25	2.25	2.9

Values are means of four replicates ± standard errors.

Table 3.1.1. Concentrations of copper (µg g^{-1} dry weight) in grains from spikes of tillers of eight Canadian spring wheat cultivars.

Treatment		Katepwa	Roblin	Park	Laura	Conway	Oslo	Columbus	Biggar
-Cu	1990	2.2 ± 0.5	2.0 ± 0.6	1.3 ± 0.2	2.1 ± 0.7	2.2 ± 0.5	1.5 ± 0.4	1.6 ± 0.4	1.8 ± 0.7
	1991	1.0 ± 0.2	1.2 ± 0.3	1.5 ± 0.2	1.7 ± 0.5	1.1 ± 0.3	1.6 ± 0.3	2.0 ± 0.4	2.2 ± 0.3
	Mean	1.60	1.60	1.40	1.90	1.65	1.55	1.80	2.00
+Cu	1990	1.7 ± 0.8	1.4 ± 0.8	2.4 ± 0.4	2.7 ± 2.2	2.2 ± 0.6	2.8 ± 0.7	1.7 ± 0.7	2.6 ± 0.5
	1991	2.0 ± 0.2	2.1 ± 0.5	1.4 ± 0.3	2.4 ± 0.5	2.0 ± 0.3	1.8 ± 0.3	1.8 ± 0.4	2.6 ± 0.5
	Mean	1.85	1.90	1.90	2.55	2.05	2.30	1.75	2.6

Values are means of four replicates ± standard errors.

Cu concentration in the leaf blades, showed lower levels of Cu in the kernels in the second season than in the first season. An average of 1.6 and 2.4 $\mu g\ g^{-1}$ Cu in the kernels from the main stems was observed for cvs. Park and Oslo, respectively, and from the tillers an average of 1.4 and 1.6 $\mu g\ g^{-1}$, was detected, on plants not treated with Cu.

3.3.5 Correlation between copper concentration in the youngest fully emerged leaves, and grain yield and yield components.

Correlations between Cu in the YFEL and yield, and some yield components were calculated in an attempt to find the stage at which Cu had direct effects on the yield of wheat. Positive correlations (Table 3.2.0 and Figure 3.2.0) were found between the grain yield and Cu in the YFEL (r = 0.79*) at Zadoks growth stage 25 , and (r = 0.76*) at Zadoks growth stage 39 from -Cu treatment in the first year of the trial. In the second year of the trial, positive correlation (r = 0.90*) was observed at Zadoks growth stage 39 (Flag leaf stage). Significant correlation (r = 0.89*) was also observed between Cu in the YFEL from plants supplied with Cu and grain yield, at Zadoks growth stage 39 in the first growing season.

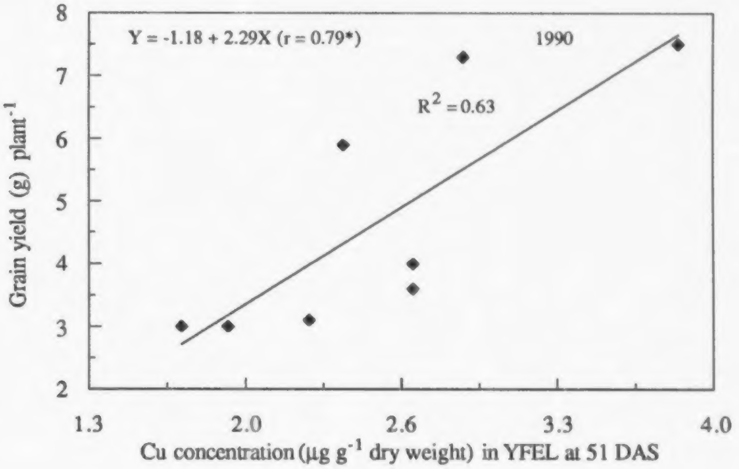
Positive correlations (Figure 3.3.0) (r = 0.79*) between Cu concentration in the YFEL and the number of grains spike^{-1} from the spikes of the main stems, tillers (r = 0.87*) were observed in the first year. In the second year, significant (r = 0.78*) correlation was observed on tillers at Zadoks growth stage 39 on plants not supplied with Cu.

The relationship between percent fertile florets and Cu concentration (Figure 3.4.0) showed positive correlations (r = 0.74*) at 12, (r = 0.74*) at 25, and (r = 0.75*) at 39 at Zadoks growth stages for main stems. For tillers, significant positive relationships were observed (r = 0.73*) at Zadoks growth stage 12, (r = 0.83*) at Zadoks growth stages 25 and 39 from -Cu treatment. In the second season, significant positive correlations were observed (r = 0.94**) for main stems and (r = 0.96**) for tillers at Zadoks growth stage 39.

Table 3.2.0. Simple correlations (*r*) of Cu concentrations in YFEL with mean grain yield, grains spike^{-1} for main stem and tillers, and percent fertile florets spike^{-1} for main stem tillers, from -Cu and +Cu treatments.

		Grain yield plant^{-1}		Grains spike^{-1}				Percent fertile florets spike^{-1}			
				Main stems		Tillers		Main stems		Tillers	
DAS (Zadoks stage)	Year	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu
21 (12)	1990	-0.05	0.64	-0.26	-0.06	-0.04	0.13	-0.43	-0.19	-0.11	-0.03
	1991	0.67	0.27	0.38	-0.19	0.46	-0.03	0.74*	0.19	0.73*	0.58
28 (22/23)	1990	0.52	0.61	-0.24	-0.07	0.21	0.13	0.13	-0.10	0.50	0.14
	1991	-0.26	0.39	-0.23	-0.17	-0.28	0.16	-0.48	-0.05	-0.43	0.42
35 (24)	1990	0.42	0.91	-0.19	0.38	0.15	0.58	0.18	0.48	0.46	0.60
	1991	0.26	0.42	-0.05	0.02	0.09	0.31	0.39	0.17	0.46	0.49
42 (25)	1990	0.79*	0.67	0.28	0.05	0.55	0.32	0.74*	0.42	0.83*	0.56
	1991	0.14	0.37	-0.07	-0.24	0.05	0.10	0.29	0.14	0.38	0.58
51 (39)	1990	0.76*	0.89**	0.79*	0.61	0.87**	0.76*	0.75*	0.72*	0.83*	0.82*
	1991	0.90**	-0.05	0.66	0.16	0.78*	0.08	0.94**	0.32	0.96**	0.03

*, ** = significant ($P \leq 0.05$) and ($P \leq 0.01$) respectively.



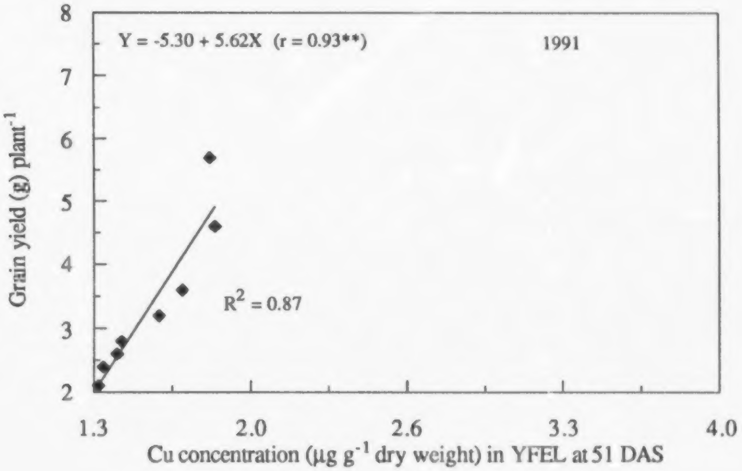


Figure 3.2.0. Relationship between the grain yield of eight Canadian spring wheat cultivars and Cu concentration in the YFEL at 51 days after sowing (DAS) sampled from the -Cu treatment for 1990 and 1991.

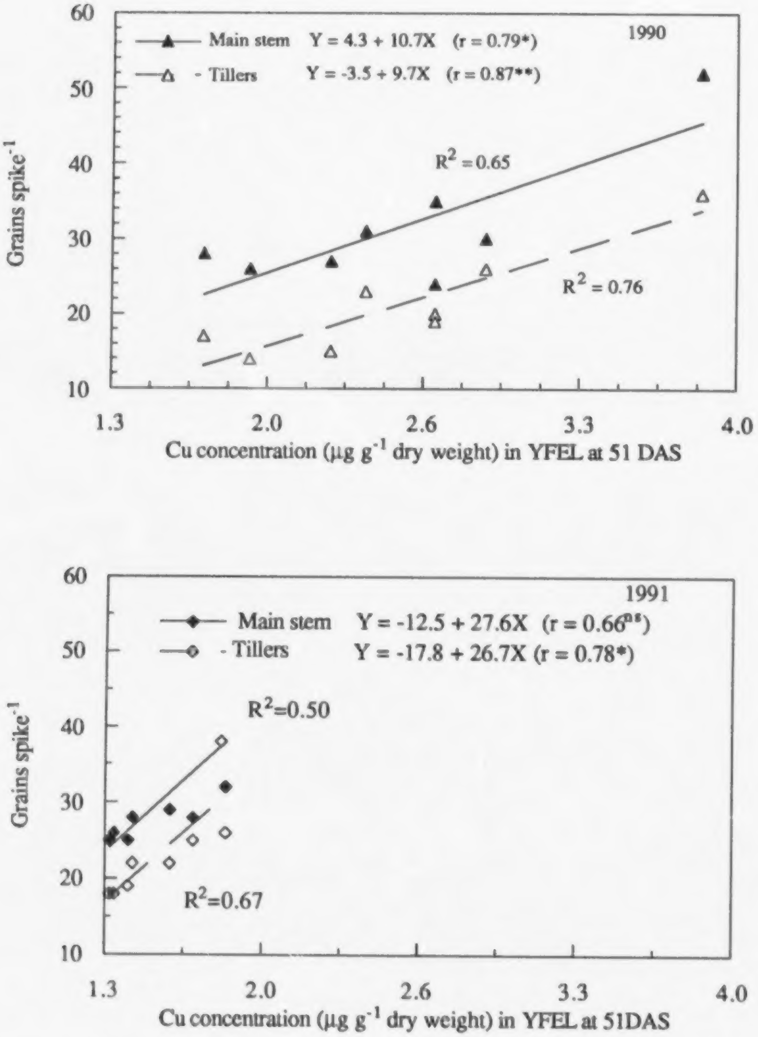


Figure 3.3.0 Relationship between mean number of grains spike^{-1} of main stems and tillers of eight Canadian spring wheat (*T. aestivum*) cultivars and Cu concentration in the YFEL at 51 days after sowing (DAS). The leaves were sampled from the -Cu treatment.

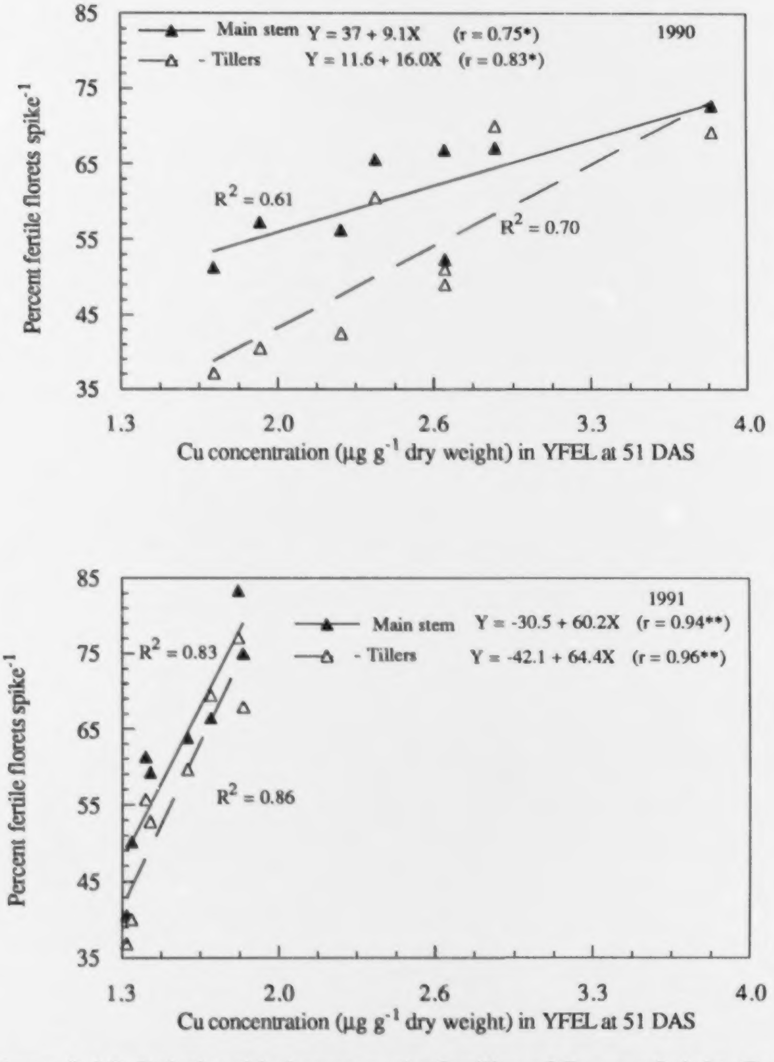


Figure 3.4.0 Relationship between the fertility of florets of eight Canadian spring wheat cultivars (*T. aestivum*) and Cu concentration (µg g^{-1} dry weight) in the YFEL at 51 days after sowing (DAS). Samples were obtained from the -Cu treatment.

3.4.0 DISCUSSION

Symptoms.

Of the eight cultivars studied, the expressed symptoms of Cu-deficiency varied with the cultivars and seasons, and were consistent with those reported by Gartrell *et al.* (1979a), Graham and Nambiar (1981), and Gartrell (1981). Foliar Cu-deficiency symptoms were observed earlier in 1990 than in 1991. The symptoms were more pronounced in the second season, most likely due to the influence of soil moisture deficit on Cu nutrition. The intensity of foliar deficiency symptoms which were mainly necrosis and rolling of the youngest leaves on cvs. Katepwa, Roblin and Park diminished as the plant matured in the 1990 growing season. This was probably due to sufficient soil moisture for most of the growing period which might have increased the availability of Cu^{2+} ions for plant roots in the top soil. Secondly, as the plants progressed in growth and development, the sensitivity to Cu-deficiency may have changed and it depend on the cultivars and environment. Cultivars Roblin, Park and Katepwa showed a decrease in symptoms with plant growth during the first year of trial, but in the second year, the intensity of symptoms increased with the plant growth. However, the appearance of deficiency symptoms on +Cu treatments on cvs. Park, Katepwa and Roblin in 1990 indicated that wheat cultivars have different requirements for Cu.

Analysis of variance over time.

The test cultivars differed significantly in uptake and accumulation of Cu in the YFEL. This suggests that the efficiency for Cu uptake differs among the wheat cultivars. Significant effects due to Cu showed that the increase in Cu in the YFEL was due to Cu supplied to the soil. Although the study was conducted in the same site, Cu concentration in the YFEL of the test cultivars varied significantly with the year. During growth and developmental processes, Cu concentration in the leaves differed significantly between the two years and this provided evidence that the status of Cu in the wheat plants, changes with growth stage. In this study, year to year variation influenced Cu nutrition of the tested cultivars, consequently enhanced differences in Cu concentration in the YFEL. From the results, Cu concentration in the YFEL depended on cultivar, growth stage at sampling time and year.

Cu concentrations in the YFEL.

The use of tissue analysis as a diagnostic tool for detection of Cu-deficiency indicates the status of Cu in the plant at the time of sampling. In this study, the YFEL were used for plant Cu analysis in order to reduce physiological differences among the test cultivars due to the age and part of the plant sampled, and Cu treatment. Copper content in the YFEL decreased gradually with the maturity of the plants (Appendix 3.0.0, Figures 3.1.0-3.1.1). This observation was consistent with similar patterns of copper content detected in the leaf blades of oats as the plant matures (Williams and Moore, 1952), for wheat (Loneragan, 1981) and for subterranean clover (Reuter *et al.*, 1981b). Several explanations could be proposed for the decline of Cu with the progressive aging of plants. First, Cu interacts with N in the leaves and as a result, reduces its mobility within the plant system in wheat until the senescence stage (Hill *et al.*, 1979). Secondly, Cu has been suggested to be a phloem-immobile micronutrient (Graham, 1991). The test cultivars showed genetic variation in their capability for Cu uptake and translocation to the leaves. This agrees with the findings of Smilde and Henkens (1967), Nambiar (1976a), and Graham (1984) who described differences in the genetic capability of wheat cultivars for the uptake of Cu and for grain production on Cu-deficient soils. Those cultivars of wheat that have high demand for Cu during growth processes (for instance cv. Park and Oslo) showed the deficiency symptoms on the young developing organs at Zadoks growth stage 22 and 24.

In this study, the supply of Cu increased the level of Cu in the leaf blades of all cultivars. However the critical Cu concentration, based on the deficiency symptoms for the two seasons differed, with a higher level in the first than in the second growing season for all cultivars (Appendix 3.0.0). Although the differences in Cu uptake in the two years showed that seasons have an influence on Cu nutrition, Cu-use efficiency still varied between cultivars.

The appearance of Cu-deficiency symptoms on leaves of some cultivars and not in others, although they had more or less the same Cu concentrations (Appendix 3.0.0 Figure 3.1.0-3.1.1), suggest that the test cultivars had different Cu requirements. This finding differed from the suggestion of Snowball and Robinson (1984) that cultivars do not differ in their internal Cu requirements. Cultivar Columbus did not show deficiency symptoms although the Cu levels in the YFEL ranged between 5.4 ± 0.2 and 2.6 ± 0.4 $\mu g\ g^{-1}$ (-Cu) for the two seasons at Zadoks growth stage 22.

Several physiological mechanisms may be responsible for cultivar differences in Cu-efficiency. Nutrient efficiency may be expressed in terms of absorption, uptake, translocation, distribution within the plant systems, utilization, mobilization and reutilization efficiency (Clark, 1990). Cultivar Katepwa showed the highest Cu uptake and translocation to the YFEL and showed high Cu requirement. Cultivars Oslo and Park were poor in Cu uptake. Because of small differences in Cu in YFEL at Zadoks growth stage 39, Cu-efficiency for the test cultivars was mainly determined by the efficiency of redistribution and reutilization of Cu, from the booting to senescence stage. Gerloff and Gobelman (1983) argued that weak redistribution of elements would result in element retention in old inactive leaves instead of being utilized in young growing tissues. Since Cu is used in mobilization and remobilization of carbohydrates at the senescence stage (Brown and Clark,1977) it was evident in this study that, except for cv. Biggar, cultivars were poor in reutilizing Cu at later stages of growth. Because of this, Cu concentration in the flag leaves should not be used to indicate critical Cu concentrations, in agreement with the argument by Nambiar (1976b) that Cu content in the flag leaves does not properly represent Cu status of the whole plant.

Supply of Cu to the plants increased Cu content in the grains. In the first growing season, the accumulation of Cu in the grains from the spikes of main stems was higher than its accumulation for grain from tillers for cvs. Katepwa, Roblin, Park, Laura, Oslo, Columbus and Biggar, but this response was not the same in the subsequent season for cv. Oslo and Columbus. Cultivar Park did not show differences in Cu levels in the grains from +Cu and -Cu treatments from the tillers. This could be due to the low rate of uptake, combined with a higher demand for Cu.

Correlation between grain yield, the number of grains spike^{-1}, floret fertility and Cu concentrations in YFEL.

Positive correlations between Cu concentration in the YFEL at Zadoks growth stage 25 and 39 for grain yield, grain number and floret fertility indicated that the increase in Cu in the YFEL increased grain yield, grain number spike^{-1}, and percent floret fertility. Yield was improved because of improved fertility and consequently, the number of grains spike^{-1}. In the first year of the trial, significant correlation at Zadoks growth stage 25 could be attributed to adequate moisture supply, then improving Cu availability in the soil (Grundon, 1991), and enhancing the uptake and reutilization of Cu, at least for most cultivars. It seemed that when Cu is supplemented, Cu supply becomes sufficient, accounting for non significant correlations in the +Cu treatment. The relationship between the grain yield, the number of grains spike^{-1} and Cu concentration in the YFEL from -Cu treatment was linear. This suggests that Cu concentration in the leaves was within the deficiency range and had not reached the tolerance level (Berry and Wallace, 1981). The relationship between percent fertile florets spike^{-1} and Cu concentration in the YFEL at Zadoks growth stage 39 showed a curve-linear relationship.

3.5.0 CONCLUSION.

From this study, it was evident that Cu concentration in the YFEL decreased as the wheat plants matured. Secondly, critical Cu concentration, in the plant leaves depends on the cultivar, year, growth stage of sampling, and Cu status in the soil. The use of tissue analysis technique needs to be complimented with visual deficiency symptoms and soil analysis technique. Tissue analysis technique can only be used to detect level of one element at a time and the level of Cu detected depends on the environment, organ of the plant sampled and stage of sampling. Lastly, Cu level in the leaves at Zadoks growth stage 39 was significantly related to grain yield, to floret fertility and grain set.

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CHAPTER 4

RESPONSE TO COPPER OF FOUR CANADIAN SPRING WHEAT (*T. aestivum* L.) CULTIVARS GROWN UNDER GREENHOUSE CONDITIONS

4.0.0 INTRODUCTION

Soils of Canada have been reported to be Cu-deficient in Alberta (Penney *et al* , 1988), Saskatchewan, (Kruger *et al* ., 1985; Karamanos *et al*., 1986), and Prince Edward Island (Gupta and MacLeod, 1970). Copper deficiency may be natural or induced. Natural Cu-deficiency results from insufficient Cu in the soil forming minerals e.g. in pumice and in soils high in organic matter (Pinkerton, 1967; Kubota and Alloway, 1972). Copper forms insoluble ion complexes in soils with organic matter greater than 10% (Alloway and Alison, 1984). Induced Cu-deficiency is due to the excess supply of other nutrient elements which causes imbalance (Murphy *et al*.,1981). Antagonistic absorption has been reported between Cu and Zn in rice (*Oryza sativa*) and tomato (*Lycopersicon esculetum*) (Bowen, 1987). High nitrogen fertilizer application in wheat also induces Cu-deficiency by encouraging fast plant growth in wheat, hence increasing the demand for Cu (Chaudhry and Loneragan, 1970; Brennan 1991). Low soil moisture also reduces availability of Cu for plants (Grundon, 1991). Soil fertility management is an important aspect in crop production. The development of new cultivars under high levels of fertilizer input results in cultivars which may have high demand for nutrients. The response of wheat to Cu treatments can be elaborated by dose response studies (Berry and Wallace, 1981).

The objective of this study was to determine the level of copper in the soil that would cause differential response to Cu supply in four Canadian spring wheat cultivars.

4.1.0 MATERIALS AND METHODS

The four spring wheat (*T. aestivum* L.) cultivars Biggar, Park, Katepwa and Oslo used in the test were selected on the basis of their performance from the first year of field trials at Stony Plain, Central Alberta. Cultivar Biggar (late in maturity), and Oslo (medium in maturity) are awned and semi-dwarf, while cv. Park (early in maturity), and Katepwa (medium in maturity) are tall and awnless.

The characteristics of the soil that was used for this study are described in section 2.1.0 (Table 2.0.0). A bulk sample (0-15 cm depth) of orthic dark grey Chernozem soil known to be deficient in Cu was obtained from Stony Plain from a farmer's field at the end of the growing season in 1990. The soil was pulverized mechanically to break down large clods, and was air-dried at room temperature for several weeks. Before using the soil, it was sterilized in an autoclave at 132 ^{o}C and 1.55 kg cm^{-2} for 60 min. Three-kilogram samples of dry soil were weighed and spread evenly on a 0.6 × 0.6 m polyethylene sheet at about 1 cm thick.

A basal fertilizer composition of 350.0 ($Ca(NO_3)_2.4H_2O$), 83.3 (NH_4NO_3), 231.3 (KH_2PO_4), and 231.3 mg (KNO_3) was supplied in solution form to each sample and mixed in thoroughly. Each soil sample received 20 ml of nutrient solution which supplied an equivalent of 70 kg (N) and 45 kg (P) ha^{-1}. Copper sulphate ($CuSO_4.5H_2O$) was supplied in a solution form at 20 ml pot^{-1} in order to supply Cu at the following rates: 0 (Cu_0), 4.3 (Cu_1), 8.5 (Cu_2), 25.7 (Cu_3), 42.7 (Cu_4), 56.6 (Cu_5), 76.7 (Cu_6), 110.0 (Cu_7), and 145.0 (Cu_8) µg kg^{-1} soil. Each of the soil samples was thoroughly mixed and used to fill 20-cm diameter non-draining plastic pots. Pots were washed in a solution of nitric acid and rinsed with distilled water to minimize elemental (Cu) contamination. Ten seeds of each cultivar were sown in each pot. At 10 days after sowing, seedlings were thinned to the five most uniform. Pots were adequately supplied with distilled water throughout the growing period until all the plants were mature for harvesting. The experiment was conducted in the greenhouse with an average day and night temperature cycle of 21 and 17 ^{o}C, respectively. The relative humidity (RH) was about 65-70% and the average light intensity was 155 ± 0.16 µmol m^{-2} sec^{-1} as measured by an integrated quantum radiometer RI-188. Light was supplemented with incandescent bulbs placed at a height of 115 cm directly above the pots throughout the growing period. The experimental plants received a photoperiod of 16 hours day^{-1}.

Experimental design and statistical analyses.

The experiment was a randomized complete block factorial design with four replicates, four wheat cultivars, and nine Cu treatments. At harvesting time, the main spikes were separated from those of the tillers. The data recorded included the number of grains spike^{-1} from the spike of the main stems and tillers, and the grain yield pot^{-1}. Analysis of variance was performed using a random effects model on a SAS program (SAS Inst., 1989). The means of cultivars were separated using a least significance difference statistical test at the 95% confidence level.

4.2.0 RESULTS.

4.2.1 Symptoms.

Cultivar Park growing in the no copper treatment (Cu_0) was reduced in growth and vigour starting 18 days after sowing. Observed symptoms included leaf dieback from the apex of the young emerging leaf. These symptoms were observed on both Cu_0 and Cu_1 treatments. The young unfolded leaves of cv. Park and Oslo were chlorotic, and the midrib split, on Cu_2 and Cu_3 treatments at 25 DAS. Cultivar Park showed Cu-deficiency symptoms on the Cu_4 treatment, whilst cv. Katepwa showed Cu-deficiency symptoms at all levels of Cu supply. Park and Katepwa showed late maturity characteristics on the Cu_0, Cu_1, Cu_2, Cu_3 and Cu_4 treatments. Cultivar Biggar did not show Cu-deficiency symptoms for any of the Cu treatments. Cultivar Park, Oslo, and Katepwa were late maturing in treatments Cu_0-Cu_2. The florets of cv. Oslo and Park were devoid of grains, an indication of sterility caused by Cu-deficiency. These symptoms were typical of those described by Gartrell *et al*. (1979), Graham (1975), and Dell (1981), and were seen in the field trial.

4.2.2 Analyses of variance for grain yield and the number of grains spike^{-1}.

Results (Table 4.0.0) showed significant ($P \leq 0.05$) effects due to cultivar and copper for grain yield, number of grains spike^{-1} of main stems and number of grains spike^{-1} of tillers with coefficient of variation of (cv) of 25.9%, 26.0% and 27.1%, respectively. No significant interaction effect was observed between cultivar and copper.

Table 4.0.0. Analyses of variance for grain yield and the number of grains spike^{-1}

		Mean squares		
		Grain yield	Number of grains spike^{-1}	
Source	df	g pot^{-1}	Main stems	Tillers
Rep	3	2.8	20.4	119.8
Cultivar	3	152.9**	2778.0**	1661.9**
Cu	8	68.7**	242.7**	174.5**
Cultivar × Cu	24	4.7	25.1	25.0
Error	105	6.4	42.6	19.7
cv (%)		25.9	26.0	27.1

*, ** significant ($P \leq 0.05$), and ($P \leq 0.01$), respectively..

Among the four test cultivars, cv. Biggar showed the highest ($P \leq 0.05$) mean grain yield at all levels of Cu, with the highest yield in the Cu_8 treatment (Table 4.1.0). Cultivars Park and Oslo showed an increase in grain yield of 400% of the control. The highest responses were observed on Cu_8 treatment for cvs. Biggar and Park, and there was no significant difference in grain yield between these two cultivars. On the Cu_0 treatment, cv. Biggar significantly produced the highest grain yield while Oslo produced the lowest. Cultivar Katepwa showed inconsistency of response to Cu treatment. The maximum grain yield was observed with the Cu_4 and Cu_7 treatments. For grain yield, significant ($P \leq 0.05$) differences among Katepwa, Park and Oslo were not found except for the Cu_8 (145 µg kg^{-1} soil) treatment. However, the three cultivars differed significantly in yield from cv. Biggar. The mean separation of cultivars showed significant differences between cultivars but there was no significant between Park and Katepwa.

The number of grains spike^{-1} for cv. Biggar (Table 4.2.0) increased with the increase of Cu levels, reaching a maximum for the Cu_8 treatment. Both on spikes from the main stems and the tillers, cv. Katepwa showed an increase in the number of grains spike^{-1} with an increase of Cu applied, reaching a maximum with the Cu_4 treatment.

Table 4.1.0. Effect of copper treatments on grain yield of four Canadian spring wheat cultivars.

	Cu supplied to soil (µg kg^{-1} soil)									
Cultivar	Cu_0 (0)[†]	Cu_1 (4.3)	Cu_2 (8.5)	Cu_3 (25.7)	Cu_4 (42.7)	Cu_5 (56.6)	Cu_6 (76.7)	Cu_7 (110)	Cu_8 (145)	Mean Cu_{0-8}
Biggar	7.8 a	9.1 a	9.5 a	10.7 a	12.4 a	10.5 a	11.8 a	13.3 a	14.2 a	11.0 a
Katepwa	5.0 b	6.8 ab	6.6 b	8.6 ab	10.6 ab	6.2 b	9.9 ab	10.3 ab	8.2 b	8.0 b
Park	2.8 c	5.3 b	6.2 b	8.3 ab	7.6 b	7.2 ab	8.4 b	9.0 b	13.2 a	7.6 b
Oslo	1.7 c	5.9 b	5.1 b	6.2 b	8.6 b	5.4 b	7.1 b	6.8 b	8.6 b	6.1 c
LSD (0.05)	1.8	2.7	2.0	3.7	3.0	3.5	3.4	3.9	4.2	0.9
Mean	4.3	6.8	6.9	8.4	9.8	7.3	9.3	9.9	11.1	

Means with the same letter within the column are not significantly different.

Cultivar Park showed an increase in grain number spike^{-1} with increased supply of Cu. Maximum mean grain numbers on the main stems were observed at Cu_8 treatment for cv. Park, whilst for cv. Oslo the maximum mean grain numbers from the main stem and tillers was observed for the Cu_4 and Cu_5 treatment, respectively. The four test cultivars showed an increase in grain yield and grain numbers as Cu supply was increased. The means for the number of grains spike^{-1} of cultivars were significantly different (Table 4.2.0 and 4.3.0) The grain yield and the number of grains spike^{-1} increased with the increase of the level of Cu in the soil (Figures 4.0.0 and 4.1.0)

Table 4.2.0. Effect of copper treatments on the number of grains spike^{-1} from main stems of four Canadian spring wheat cultivars.

	Cu supplied to soil (μg kg^{-1} soil)									
Cultivar	Cu_0 (0)	Cu_1 (4.3)	Cu_2 (8.5)	Cu_3 (25.7)	Cu_4 (42.7)	Cu_5 (56.6)	Cu_6 (76.7)	Cu_7 (110)	Cu_8 (145)	Mean Cu_{0-8}
Biggar	26.4 a	31.5 a	35.5 a	38.5 a	39.5 a	39.0 a	37.3 a	41.5 a	42.0 a	36.8 a
Katepwa	20.3 a	22.7 ab	27.5 ab	28.2 ab	29.5 b	27.2 b	26.0 b	26.8 b	25.5 b	25.9 b
Park	13.4 ab	20.8 ab	22.5 bc	19.6 bc	21.2 c	21.7 bc	22.7 bc	23.1 bc	28.6 b	21.5 c
Oslo	5.6 b	15.6 b	14.5 c	13.6 c	21.9 c	14.5 c	17.4 c	16.0 c	20.6 b	16.1 d
LSD (0.05)	13.6	12.7	8.5	11.3	6.6	9.4	6.0	9.4	5.2	3.1
Mean	16.4	22.7	25.0	24.9	28.0	25.6	25.7	26.8	29.2	

Means with the same letter within the column are not significantly different.

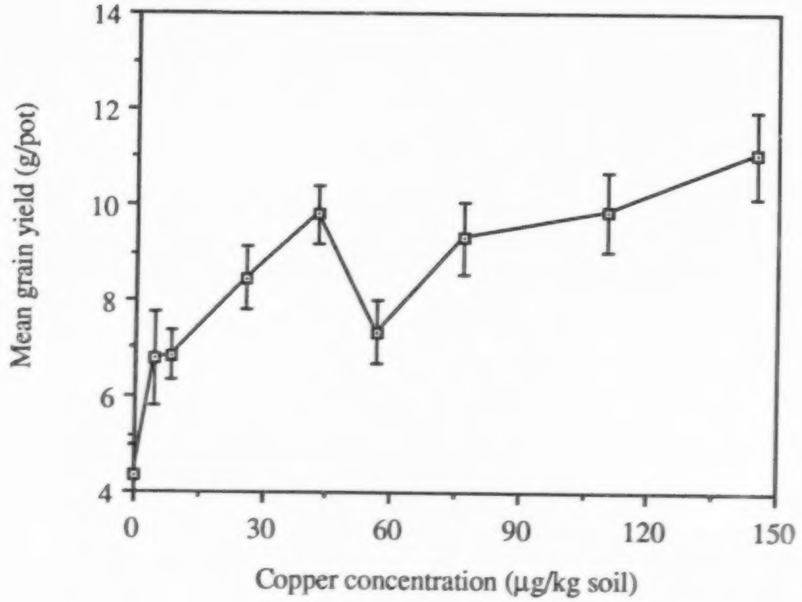

Figure 4.0.0. Response to copper supply of grain yield of four Canadian spring wheat (*Triticum aestivum* L.) cultivars grown under greenhouse conditions. Error bars represent standard errors

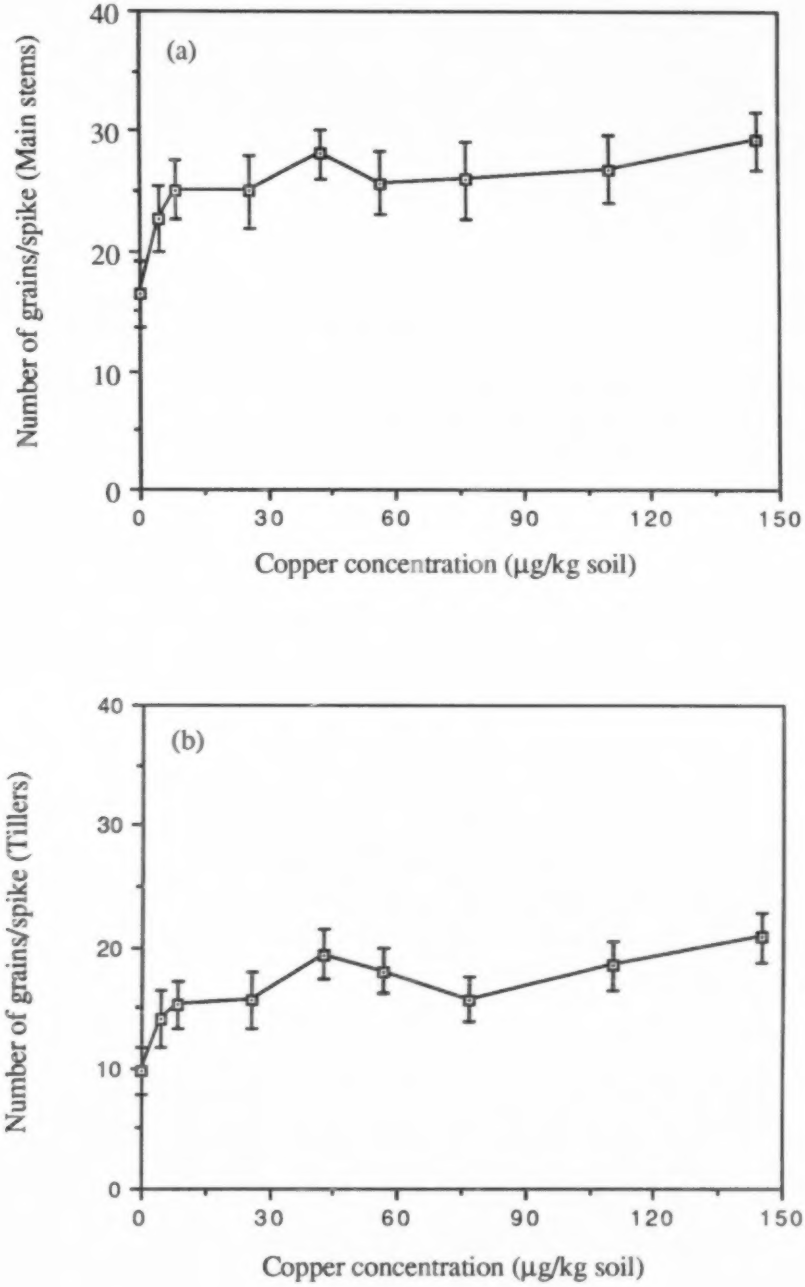

Figure 4.1.0. Response to copper supply of number of grains spike^{-1} of (a) main stem and (b) of tillers of four Canadian spring wheat (*Triticum aestivum* L.) grown under greenhouse conditions. Error bars represent standard errors.

Table 4.3.0. Effect of copper treatments on the number of grains spike^{-1} from tillers of four Canadian spring wheat cultivars.

	Cu supplied to soil (µg kg^{-1} soil)									
	Cu_0	Cu_1	Cu_2	Cu_3	Cu_4	Cu_5	Cu_6	Cu_7	Cu_8	Mean
Cultivar	(0)	(4.3)	(8.5)	(25.6)	(42.6)	(56.6)	(76.6)	(110)	(145)	Cu_{0-8}
Biggar	16.5 a	22.8 a	23.7 a	24.6 a	29.0 a	25.0 a	22.2 a	27.9 a	32.1 a	24.8 a
Katepwa	11.6 b	11.8 b	19.1 a	18.7 ab	20.6 b	16.4 ab	17.7 b	21.5 a	19.2 b	17.4 b
Park	6.8 c	12.4 b	10.5 b	13.8 bc	17.4 b	15.5 ab	16.2 b	17.2 ab	21.7 b	14.6 c
Oslo	4.1 c	9.2 b	7.8 b	5.5 c	10.5 c	15.1 b	6.6 c	7.4 b	10.2 c	8.4 d
LSD 0.05	3.4	5.2	7.2	8.4	5.5	9.6	4.2	10.8	5.1	2.1
Mean	9.6	14.1	15.3	15.7	19.4	18.0	15.7	18.5	20.8	

Means with the same letter within the column are not significantly different.

4.3.0 DISCUSSION

As the wheat plant grows and develops, its sensitivity to Cu deficiency may change depending on the cultivar. The four test cultivars showed varying symptoms of Cu deficiency at different concentrations of Cu treatment. The observed symptoms were consistent with those previously described (Gartrell *et al.*, 1979; Graham and Nambiar, 1981; Alloway and Alison, 1984). The abortion of spikelets and sterility of florets of cv. Oslo significantly affected the grain number and grain yield production. No deficiency symptoms were observed on cv. Biggar. The induced lateness in maturity of cultivar Park and Oslo suggested impaired Cu movement at the senescence stage, which resulted in poor reutilization and distribution of Cu (Clark, 1990; Clark, 1983). It has been reported that cultivars that are inefficient in redistribution and reutilization of Cu at the senescence stage have poor carbohydrate mobilization and remobilization (Brown, and Clark 1977).

Significant ($P \leq 0.01$) differences among the four test cultivars indicated that the response to Cu depends on the genotype. Non significant cultivar × Cu interaction was due to high coefficient of variation observed on grain yield and the number of grains spike^{-1}. Cultivar Biggar showed the highest Cu-efficiency among the four test cultivars since it showed the lowest response to Cu, but had significantly higher yield than other cultivars. Cultivars Park and Oslo responded significantly to Cu supply as compared to the control (Cu_0). However, the grain yield of Park and Oslo did not exceed that of cv. Biggar. Gupta and MacLeod (1970) observed a grain yield response of 500% when Cu was applied to the wheat cv. Opal at the rate of 0.5 mg kg^{-1} soil, under greenhouse conditions. In this study, cv. Oslo responded to Cu by increasing grain yield by 420% when Cu was supplied at the rate of 42.6 μg kg^{-1} soil. Cultivar Park responded positively to Cu and produced about the same yield as cv. Biggar on soil supplied with Cu at the rate of 145 μg kg^{-1} soil. This could suggests that cv. Park has a high demand for Cu, which may be related to its fast growth rate, and early maturity. Cultivar Park and Katepwa did not show significant ($P \leq 0.05$) differences in response to Cu supply except for the Cu_8 (145 μg kg^{-1} soil) treatment. Cultivar Oslo showed the lowest response to Cu with the lowest grain yield at all levels of Cu supplied. This suggested that cv Oslo was sensitive to Cu-deficiency, and poor in Cu uptake, and utilization. The results of this study agree with reports by Smilde and Henkens, (1967) and Graham *et al.*, (1987) which indicated that wheat cultivars differ in Cu-efficiency. The results also suggest that the Cu-efficiency may vary with cultivar as a result of differences in Cu uptake, distribution, utilization and redistribution (Clark, 1990), especially during the senescence stage.

High grain yield in cv. Biggar was related to high grain number spike^{-1} on both the main stems and tillers. The highest grain numbers were produced on Cu_8 (145 μg kg^{-1} soil) treatment. Similar responses were observed on Park and Katepwa, and this suggested that the supply of Cu was essential for grain production. Cultivar Oslo did not respond to Cu above the Cu_4 level. Generally, there was increase in grain yield as the level of Cu increased (Figure 4.0.0). However, low yield at Cu5 was probably due to low efficacy of copper sulphate supplied to the soil and interaction with soil and other nutrients.

4.4.0 CONCLUSION

In this study of dose response to Cu, cv. Biggar was relatively superior to three other cultivars in Cu-efficiency, while cv. Oslo was sensitive to Cu-deficiency. Based on the similarity of the grain yield and grain number spike^{-1}, response of the four test cultivars, the rate of 145 μg kg^{-1} soil was found to be suitable to distinguish between Cu-sensitive and Cu-efficient cultivars in pot studies under greenhouse conditions using Cu-deficient soil from Stony Plain, Alberta. From this study, it was evident that Cu_8 (145 μg kg^{-1} soil), was the rate of Cu which was suitable for use to screen for Cu-efficient cultivars in soils from Stony Plain under greenhouse conditions. On the basis of experimental results, the need to supply Cu to the soils of Stony Plain for wheat production was reconfirmed.

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CHAPTER 5

THE EFFICIENCY FOR COPPER USE OF 5A/5RL WHEAT-RYE TRANSLOCATED LINES AND WHEAT (*Triticum aestivum* L.) CULTIVARS

5.0.0 INTRODUCTION.

Copper-deficient soils are widespread world wide and Cu has been known to be one of the most limiting micronutrients in grain wheat production. Copper deficiency is most pronounced on peats and light sandy soils (Graham *et al.*, 1987), on soils with consolidated pumice and ash which originated from volcanic ash (Pinkerton, 1967), and on sandy loams (Kruger *et al.*, 1985). Most Cu-deficient soils are not completely devoid of Cu, but the inadequacy can be caused by low Cu concentrations in the soil and soil solution, by the binding of Cu in an unavailable form for the plant, by inefficient absorptive mechanisms and by low rates of translocation from roots to shoots (Maclaren *et al.*, 1973; Clark, 1983).

In severe soil Cu deficiency, the young emerging leaves of wheat wither, twist and form a 'rat tail' from the apex extending distally, and plants show stunted growth with excessive tillers (Graham *et al.*, 1981). Excessive tillering increases the number of meristems, consequently contributing to the immobilization of the limited amount of available copper (Loughman *et al.*, 1983). Stem and head melanosis symptoms have been observed and associated with Cu-deficiency on wheat cv. Park in Canada (Piening *et al.*, 1985). In most cases, marginal Cu-deficiency in the soil may not be visually observed on the plant, but grain yield losses between 10 and 100% have been reported in situations of severe Cu-deficiency in the field (Alloway and Alison, 1984; Graham, 1984). The reduction in grain yield is often mistakenly attributed to soil moisture deficit and other environmental factors, although an increase in moisture deficit on the top soil surface can also immobilize available Cu in the soil (Grundon, 1980).

In the past, application of Cu-based fertilizers on Cu-deficient soils was preferred, but recently, there has been a shift towards breeding for Cu-efficient wheat cultivars, with the aim of reducing the cost of inputs in wheat production. A "Cu-efficient cultivar" is defined as a genotype that is capable of producing high grain yield in both Cu-deficient and Cu-adequate soils (Graham, 1984). Nutrient efficiency has also been defined as the ratio of plant yield to nutrient supply (Saurebeck and Helal, 1990). Therefore, those genotypes that are Cu-efficient have a higher yield to nutrient ratio. To develop Cu-efficient cultivars, there should be a potential genetic source which is efficient to use.

Wheat (*Triticum aestivum* L.) and closely related species differ in their uptake, translocation, accumulation, and use of copper (Brown and Clark, 1977). These differences are mainly genetic, but phenotypic expressions may differ when plants are grown under different environments. Diversity on the basis of Cu uptake, translocation, distribution and use has also been observed among genotypes and cultivars, within the same plant species (Smilde and Henkens, 1967; Nambiar, 1976; Graham and Pearce, 1979; Sarc, 1981). Although there has been variability in Cu-efficiency among wheat cultivars, the genetic potential for grain yield improvement in these materials is relatively low. Consequently, wheat breeders have been compelled to look for the desirable genes from wheat-related species in order to improve on Cu-efficiency. Rye (*Secale cereale* L.) is one of the most Cu-efficient cereal crops while hexaploid triticale is intermediate compared to bread wheat and (Graham and Pearce, 1979; Susan and Graham, 1981).

Sears (1967) reported the development of wheat-rye translocated lines that had a characteristic "hairy neck", which has been used as a genetic marker for Cu-efficiency. Lines with 5RL wheat-rye translocation, with hairy peduncle, have been successfully backcrossed into wheat, and progenies with high Cu-efficiency have been recovered (Graham, 1984). The nature and background under which the 5R rye chromatin is present influences the expression of the Cu-efficiency (i.e whether substituted or translocated). 5RL/4BS wheat-rye translocated near-isogenic lines have been noted to be Cu-efficient with more than 100% grain yield increase above their non-translocated parents (Graham, 1987). Work by Podlesak *et al.* (1990) on 5RL/4BS winter wheat-rye translocated lines showed significant increases in Cu-efficiency which were attributable to low internal Cu requirements. This suggestion differed from the earlier report of Graham (1984), which indicated that 5RL/4AS and 5RL/5BS wheat-rye translocated lines had higher Cu concentrations in the leaf tissues, and higher Cu uptake from Cu-deficient soils. Schlegel *et al.* (1991) recently confirmed a high Cu-efficiency for grain yield in the winter wheat cv. 'Viking' with 5BL/5RL, by growing the cultivar on Cu deficient soil. The expressivity of Cu-efficiency is still not clearly understood. However, it has recently been reported that efficiency for micronutrients (Fe, Mn, Cu and Zn) is partly related to the exudates (phytosiderophores) released by roots of efficient genotypes into the rhizosphere (Marschner *et al.*, 1986, 1987; Marschner, 1991: Zhang *et al.*, 1991).

The objectives of the greenhouse study were: (i) to determine the efficiency of 5A/5RL wheat-rye translocated lines for Cu, and (ii) to compare the Cu use efficiencies of 5A/5RL wheat-rye translocated lines to six Canadian and three Kenyan spring wheat cultivars, on the basis of grain yield and yield components, using an orthic dark grey Chernozem Cu-deficient soil from Stony Plain, Central Alberta, known to be Cu-deficient.

5.1.0 MATERIALS AND METHODS

5.1.1 Genotypes.

Six Canadian spring wheat cultivars (*T. aestivum* L.) (Oslo, Katepwa, Roblin, Columbus, Park and Biggar), three Kenyan cultivars (K. Leopard, K. Tausi, and Kwale) and three 5A/5RL wheat-rye translocated lines (CEG-2, CEG-3 and CEG-5) were used in the greenhouse experiment. Lines CEG-2, CEG-3 and CEG-5 were selected from fourteen 5A/5RL wheat-rye translocated lines derived from the CIMMYT wheat program. Selection was done in Kenya (1987-1989) during the long rain and short rain seasons on soil lacking Cu treatment in the field. Cultivar Park is an early maturing, Cu-deficiency sensitive cultivar (Piening *et al.*, 1985). The response to Cu for the remaining Canadian wheat cultivars had not been previously determined. Kenya Tausi (a tall cultivar) and Kwale (a semi-dwarf cultivar) are medium maturing cultivars which were recently released in Kenya after being selected and tested in soils of varying copper content. Kenya Leopard is an old tall cultivar, which was developed before the inception of the application of copper oxychloride to wheat in breeding nurseries and in commercial fields.

The translocated line CEG-2 (5A/5RL-3// CHILERO (42) /3/ ALD"S"/PVN "S") is an awnless late maturing, semi-dwarf with a lax spike. Line CEG-3 (5A/5RL-3// CHILERO (42) /3/ ALD"S"/PVN "S") is an awned, tall and late maturing line, and CEG-5 (5A/5RL-1//BUC"S"/BJY"S" (42)/3/ BUC"S") is an awned, early maturing, semi-dwarf with a lax spike. All the 5A/5RL wheat-rye translocated lines have hairy peduncles. Both CEG-2 and CEG-3 have medium hair density within 3-4 cm of the peduncle, while CEG-5 has high hair density within 4-6 cm of the peduncle.

5.1.2 Soil preparation and greenhouse conditions

A bulk sample of orthic dark grey Chernozem soil (depth of 0-15 cm), was obtained from Stony Plain, Central Alberta. The soil originated from deltaic material and was deficient in Cu (Alberta Soil Survey, 1970). The field from which the soil was obtained was previously planted with barley. The soil used in the study had an organic matter content of 8.9% and the following micronutrient levels: Cu 0.48, Mn 71.21, Zn 14.05, Fe 164.60, and B 0.45 (μg (DTPA extracted) g^{-1} soil). The soil was pulverized mechanically to break up large clods, was allowed to air-dry, and was then sterilized in an autoclave at 132 ^{o}C and 1.55 kg cm^{-2} pressure for one hour. Three-kilogram samples of soil were weighed and spread on a square polyethylene sheet measuring 0.6 m by 0.6 m, to a thickness of 1 cm. Basal fertilizer dressings of 350 ($Ca(NO_3)_2.4H_2O$), 83 (NH_4NO_3), 231 (KH_2PO_4), and 231 mg (KNO_3) in solution form were applied in each soil sample. Each of the soil samples was thoroughly mixed on the polyethylene sheets and filled into 20 cm diameter non-draining plastic pots which were previously washed in a solution of nitric acid and rinsed with distilled water to minimize Cu contamination. Each sample received 20 ml of each of the nutrient solution which supplied an equivalent of 70 kg (N) and 45 kg (P) ha^{-1}. A solution of 20 ml of copper sulphate (85 mg L^{-1}) was added into the soil (+Cu). No Cu was applied to the control (-Cu). The remaining micronutrients (Mn, Zn, Fe and B) were found not to be limiting according to the results of the soil analysis, and were therefore not supplemented.

Ten seeds were sown in each pot and after emergence, seedlings were thinned to the five most uniform seedlings. The pots were adequately watered with distilled water throughout the growing period until all the plants were mature for harvesting. The experiment was conducted in a controlled environment in a greenhouse with an average day and night temperature cycle of 21 and 17 ^{o}C, respectively. Relative humidity in the greenhouse was approximately 65%, and the average light intensity was 263 $\pm$ 0.13 μmol m^{-2} sec^{-1} supplemented with incandescent lamps placed at a distance of 115 cm directly above the pots throughout the growing period. The growing photoperiod was 16 hours day^{-1}.

5.1.3 Experimental design and data analysis.

The experimental design was a completely randomized block with four replicates, 12 genotypes, and two Cu treatments. The copper treatments were applied at the rate of 145 μg kg^{-1} soil (+Cu), and the control had no copper added (-Cu). The data recorded included mean plant height from each pot and the mean number of tillers $plant^{-1}$, measured immediately before harvesting. At harvesting, spike lengths were measured and spikes from the main stems were separated from those of tillers. The mean number of florets $spike^{-1}$ and grains $spike^{-1}$ were recorded. The percentage of fertile florets $spike^{-1}$ for the main stem and tillers were computed by dividing the mean number of grains $spike^{-1}$ by the mean number of florets $spike^{-1}$ multiplied by 100. The percent Cu-efficiency for grain yield were determined by expressing the ratio of yield from -Cu to that of +Cu treatment as percent. The percentage values were arc sine transformed for each replicate to reduce the heterogeneity of variances following the method described by Gomez and Gomez (1984).

5.1.4 Statistical analysis.

Analyses of variance were carried out to estimate variance components using a nested design model, where chromosome translocations were nested within the genotypes for all the parameters. The general linear (GLM) procedure on SAS (SAS Inst., 1989) was used in the analysis. Separation of genotype means was done using Duncan's Multiple Range Test (DMRT) when there were significant ($P \leq 0.05$) genotypic effects according to the analysis of variance. Student *t*-test was used for comparing significant differences between +Cu and -Cu treatment means for each genotype for grain yield and some yield components..

5.2.0 RESULTS

5.2.1 Symptoms of copper deficiency.

Copper deficiency symptoms of necrosis, twisting of the youngest leaves, and dieback, were noted on cvs. Park, Roblin, and K. Tausi at 22 days after sowing. These symptoms were similar to those described by Graham and Nambiar (1981), and Gartrell *et al.* (1979). In contrast, cv. Oslo, Katepwa and Kwale did not show any symptoms of Cu-deficiency until 36 -39 days after sowing (six-leaf and five-tiller stage, equivalent to Zadoks scale 25); (Zadoks *et al.*, 1974). Leaf necrosis and withering symptoms of Cu-deficiency were also seen on the youngest leaves of cvs. K.Tausi and Katepwa at Zadoks growth stage 25, although the cultivars were supplied with Cu (+Cu). No Cu-deficiency symptoms were observed on CEG-2, CEG-3, CEG-5, Columbus, and Biggar on the -Cu treatments. The deficiency symptoms observed on sensitive cultivars varied with the cultivars. Stunted plant growth was noted on cultivars Kwale and Katepwa. The cultivars Oslo, Katepwa and Roblin showed necrosis, 'rat tail' and dieback on the youngest leaves

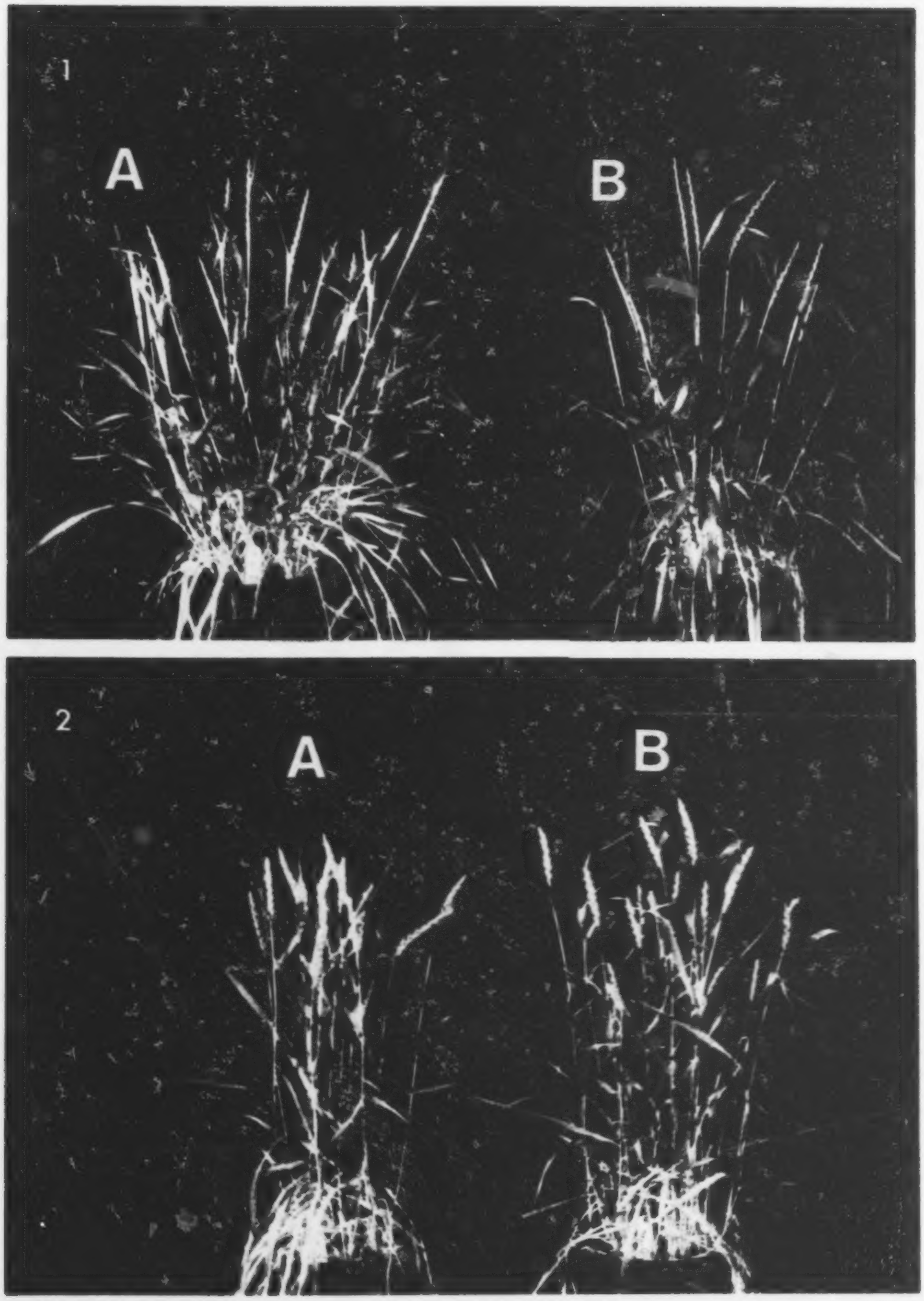

Figure 5.0.0 (1) Line CEG-2 (5A/5RL) and (2) cv. Roblin (A) copper treated (+Cu) and (B) no copper applied (-Cu). Twisting and leaf dieback of young leaves on cv. Roblin on the -Cu treatment.

(Figure 5.0.0) and spikes. Symptoms of necrosis on the flag leaves started from the apex and proceeded distally on one side of the lamina on cvs. Katepwa, Oslo and Park. The cultivar Kwale showed excessive tillering with fewer spikes produced, while cvs. Park and K. Tausi showed head and stem melanosis at the anthesis stage compared to other cultivars, K. Leopard produced fewer grains which were smaller in size, discoloured, and poorly filled, although the cultivar did not show any visual deficiency symptoms during the growing period.

5.2.2 Analysis of variance for grain yield and yield components.

The analysis of variance for grain yield and yield components (Table 5.0.0 and 5.1.0), indicated significant ($P \leq 0.01$) main effects due to translocation for grain yield, grains spike^{-1}, and percent fertile florets spike^{-1} on the main stem, and on the tillers. The number of florets spike^{-1} was not significantly ($P \leq 0.05$) affected by the presence of the translocated chromosome. There were significant ($P \leq 0.01$) effects due to the translocation for spike length and height, but the number of tillers plant^{-1} was not significantly affected. There were significant ($P \leq 0.01$) effects due to genotype for grain yield, grains spike^{-1}, florets spike^{-1} from the main stem, and percent fertile florets spike^{-1}. The number of florets spike^{-1} from tillers was significantly ($P \leq 0.05$) affected. Similarly, the effects due to genotype were significantly different ($P \leq 0.01$) for spike length, tillers plant^{-1} and height.

Significant ($P \leq 0.05$) main effects due to copper were observed on grain yield, grains spike^{-1}, percent fertile florets spike^{-1}, and florets spike^{-1}. Copper effects were significant ($P \leq 0.01$) for the spike length from the main stem. The Cu × translocation interactions were significant ($P \leq 0.05$) for grain yield and grains spike^{-1} from tillers, and the genotype × Cu interaction was significant ($P \leq 0.05$) for grain yield.

Table 5.0.0 Mean squares for grain yield and components from analysis of variance of twelve wheat genotypes (*Triticum aestivum* L.).

		Grain yield pot^{-1} g	Grains spike^{-1}		Florets spike^{-1}		Percent fertile florets spike^{-1}	
Source	df		Main stems	Tillers	Main stems	Tillers	Main stems	Tillers
Rep	3	33.4	173.9	182.6	292.0	145.5	252.7	585.2
Translocation grouping	1	571.5**	2064.0**	2069.0**	14.0	83.3	3187.9**	4600.2**
Residual (Genotypes)	10	80.4**	386.3**	151.4**	106.0**	73.6*	678.9**	468.6**
Cu	1	569.4**	1747.6**	957.0**	159.7*	26.0	2931.6**	2653.7**
Cu × Translocation grouping	1	57.0**	70.8	149.7*	22.1	13.0	86.3	383.7
Cu × Residual (Genotypes)	10	12.3*	56.7	53.7	22.6	21.0	75.4	102.3
Error	69	5.3	31.9	37.9	27.5	29.6	65.8	117.5
cv(%)		28.6	26.3	37.5	10.9	13.0	20.0	28.9

Data for percent fertile florets were transformed using arc sine.
*, ** significant ($P \leq 0.05$), and ($P \leq 0.01$), respectively.

Table 5.1.0 Mean squares for number of tillers plant^{-1}, spike length (cm), and height (cm) from analysis of variance of twelve wheat genotypes (*Triticum aestivum* L.).

		Tillers plant^{-1}	Spike length (cm)		Height (cm)
Source	df		Main stems	Tillers	
Rep	3	6.3	1.0	0.5	96.6
Translocation grouping	1	1.9	314.4**	252.7**	2027.3**
Residual (Genotypes)	10	5.4**	4.6**	4.8**	470.9**
Cu	1	1.2	3.2**	1.5	128.1*
Cu × Translocation grouping	1	1.6	1.4	0.6	85.5
Cu × Residual (Genotypes)	10	0.6	0.3	0.7	22.1
Error	69	1.2	0.4	0.6	25.9
cv (%)		20.2	6.9	9.1	8.7

*, ** significant ($P \leq 0.01$) and ($P \leq 0.05$), respectively.

5.2.3 Separation of means of genotypes.

The Duncan's multiple range test (DMRT) (Table 5.2.0) indicated significant ($P \leq 0.05$) differences between the genotypes for grain yield, grains spike^{-1}, and percent fertile florets spike^{-1}. Lines CEG-2 and CEG-3 showed significantly higher mean values than other cultivars for grain yield, number of florets spike^{-1}, and spike length although, CEG-3 demonstrated relatively lower number of tillers plant^{-1}. However, among the wheat cultivars, significant differences in grain yield pot^{-1}, grain number spike^{-1} and percent fertile florets spike^{-1} were observed between cvs. K. Leopard and Biggar. Relatively higher number of tillers plant^{-1} were observed on cv. K. Leopard compared to cv. Biggar, but Biggar demonstrated a significantly higher mean number of grains spike^{-1} and percent fertile florets spike^{-1}. Kwale and Katepwa, had high number of tillers plant^{-1}, although the number of tillers were relatively high for these two cultivars, most tillers did not bear spikes with seed. K. Tausi, Kwale, Oslo, and Katepwa showed low mean values for most of the agronomic characters, except for the number of florets spike^{-1}.

5.2.4 Efficiency for Copper use.

Of the three wheat-rye translocated lines, CEG-2 and CEG-3 produced significantly higher grain yield than the rest of the test genotypes on Cu-deficient soil in this greenhouse test, with an efficiency of 86% for CEG-2 and 70% for CEG-3 (Table 5.3.0). Line CEG-5, showed a grain yield efficiency of 127% although the grain yield was relatively lower compared to CEG-2, CEG-3 and K. Leopard. Kenya Leopard, which yielded highest among the cultivars on Cu-deficient soil (-Cu), was comparable to CEG-3 on the basis of grain yield and Cu-efficiency. Columbus showed an efficiency of 76% but produced relatively lower grain yield. K. Tausi, Kwale, Katepwa, Roblin, Oslo and Park were shown to be Cu- inefficient with relatively low grain yields and an efficiency ranging from 8-36%. All the tested genotypes responded to soil Cu treatment, with varying magnitude of grain yield response, except for CEG-5. Line CEG-2 increased grain yield by 24%,

Table 5.2.0 Comparisons of means of twelve genotypes (*Triticum aestivum* L.) for yield and yield components from the green house experiment, averaged over -Cu and +Cu treatments. (Details of genotype × Cu treatment interactions are described in tables 5.3.0 and 5.3.1).

Genotype	Grain yield pot^{-1} (g)	Florets spike^{-1}		Grains spike^{-1}		Percent fertile florets spike^{-1}		Height (cm)	Ear length (cm)		Tillers plant^{-1}
		Main stems	Tillers	Main stems	Tillers	Main stems	Tillers		Main stems	Tillers	
CEG-5¶	8.2 cd	44.5 de	40.3 abcd	23.8 c	21.3 ab	52.2 abc	53.1 a	59.9 cd	13.1 a	11.8 a	4.7 cd
CEG-2¶	15.2 a	52.3 ab	46.4 a	31.3 a	26.1 a	50.2 ab	56.2 a	62.7 bc	13.0 a	12.2 a	6.3 ab
CEG-3¶	13.3 ab	49.3 abcd	43.2 abc	33.1 a	27.5 a	66.9 a	64.1 a	77.3 a	11.6 b	10.7 b	4.6 d
K. Leopard	12.4 b	42.9 e	35.9 d	22.8 c	18.4 bc	52.1 abc	51.3 a	66.8 b	7.9 e	7.4 ef	6.7 a
K. Tausi	3.5 g	50.9 abc	43.2 abc	8.1 f	7.7 e	10.8 g	12.4 e	61.7 bc	8.9 d	8.3 d	5.4 bcd
Kwale	4.5 fg	47.3 bcde	39.7 cbd	10.0 ef	10.1 e	18.5 fg	24.2 de	42.5 f	7.8 e	7.1 f	6.5 ab
Oslo	6.7 cdef	50.0 abcd	45.0 ab	20.4 cd	14.5 cbde	39.8 cd	30.3 bcd	53.5 e	9.9 c	9.5 c	4.5 d
Katepwa	2.9 g	43.2 e	40.2 abcd	15.5 de	10.8 de	34.2 de	24.4 de	55.2 de	8.0 e	7.4 ef	5.9 abc
Roblin	5.3 efg	47.8 abcde	43.0 abc	13.4 ef	10.8 de	24.7 ef	20.2 de	50.5 e	8.2 e	7.6 def	4.7 d
Columbus	6.7 def	45.4 cde	37.9 cd	22.9 c	17.1 bcd	50.7 bc	46.3 ab	59.7 cd	7.7 e	7.0 f	5.6 abcd
Park	7.3 cde	49.4 abcd	41.0 abcd	25.0 bc	12.7 cde	49.5 bc	30.9 cde	60.9 c	8.9 d	8.3 d	5.4 bcd
Biggar	9.7 c	53.6 a	44.3 abc	30.2 ab	21.3 ab	54.9 abc	45.1 abc	53.4 e	8.1 e	8.0 de	4.9 cd

Means followed by the same letter within the column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
¶ 5A/5RL wheat-rye translocation lines.

Table 5.3.0 The effects of copper on grain yield and Cu-efficiency of twelve spring wheat genotypes (*T. aestivum* L.) grown on orthic dark grey Chernozem Cu-deficient soil in the greenhouse.

Genotype	Grain Yield g pot^{-1}		Efficiency† %	Percent of Park	
	-Cu§	+Cu§		-Cu	+Cu
CEG-5¶	8.86	7.44 ns	127 a	233	68
CEG-2¶	13.53	16.82 ns	86 b	356	154
CEG-3¶	10.94	15.67 *	70 bcd	288	144
Kenya Leopard	10.24	14.64 ns	71 bc	269	134
Kenya Tausi	0.67	6.35 **	8 f	18	58
Kwale	0.82	8.17 **	10 ef	22	75
Oslo	3.92	9.50 *	42 cde	103	87
Katepwa	1.12	4.64 *	22 ef	29	43
Roblin	1.74	8.93 *	21 ef	46	82
Columbus	5.80	7.60 ns	76 bc	153	70
Park	3.80	10.90 *	36 ef	100	100
Biggar	5.14	14.36 *	37 def	135	132
			Lsd 0.05 33.8		

† Cu-efficiency of genotypes calculated from: (-Cu/+Cu)100. Detailed analysis of variance is shown in appendix 5.1.0

*, ** - ($P \leq 0.05$) and ($P \leq 0.01$), and ns - not significant.

§ means of four replicates.

¶ 5A/5RL wheat-rye translocated lines.

Table 5.3.1. Effects of copper on number of grains spike^{-1} and percent fertile florets spike^{-1}.

Genotype	Grains spike^{-1}				Percent fertile florets spike^{-1}			
	Main stem		Tillers		Main stem		Tillers	
	-Cu§	+Cu§	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu
CEG-5¶	24	24 ns	21	22 ns	54	53 ns	51	55 ns
CEG-2¶	28	34 ns	26	26 ns	55	65 ns	56	56 ns
CEG-3¶	28	38 ns	24	31 **	57	78 **	57	71 *
K. Leopard	21	24 ns	17	20 ns	49	57 ns	48	54 ns
K. Tausi	4	12 ns	4	12 ns	10	21 ns	9	24 ns
Kwale	6	13 ns	5	15 ns	15	27 ns	16	38 ns
Oslo	14	27 **	9	20 **	27	54 *	20	44 *
Katepwa	8	23 ns	8	14 *	23	48 *	26	32 ns
Roblin	5	21 ns	6	16 *	11	44 *	13	36 ns
Columbus	22	23 ns	17	17 ns	50	54 ns	46	47 ns
Park	21	29 ns	8	17 ns	44	58 ns	18	42 ns
Biggar	23	38 *	12	31 *	46	65 *	28	64 *

*, ** - ($P \leq 0.05$) and ($P \leq 0.01$), respectively, and ns - not-significant.

¶ 5A/5RL wheat-rye translocated lines.

§ - Means of four replicates.

CEG-3, by 43%, K. Leopard by 43% and Columbus by 31%. Biggar and Park showed a response of 179 and 186%, respectively. K. Tausi, Kwale, Oslo, and Roblin showed a grain yield response to Cu of 2-9 fold more on the +Cu than on the -Cu treatments. Therefore, the least grain response to Cu was observed on wheat-rye translocated lines compared to the wheat cultivars.

In this investigation, effects of Cu on grain yield were significant ($P \leq 0.01$) for K. Tausi, Kwale and ($P \leq 0.05$) for CEG-3, Biggar, Park, Oslo, Roblin and Katepwa (Table 5.3.0). Lines CEG-2, CEG-5, and cultivars K. Leopard and Columbus did not show significant grain yield response to Cu. This indicates a high Cu efficiency, while high significant differences between (+Cu) means and (-Cu) means for Kwale and K. Tausi indicate Cu-inefficiency. Cultivar Oslo and Biggar showed significant ($P \leq 0.05$) increase in the number of grains and the floret fertility due to Cu effects (Table 5.3.1). Significant ($P \leq 0.05$) effects due to Cu on the number of grains spike^{-1} were observed on CEG-3, cv. Oslo, Katepwa and Roblin.

5.3.0 DISCUSSION

Although copper is required in only small quantities by a plant, it is a micronutrient that plays vital roles within the plant physiological system which contribute towards growth, and grain production in wheat. The results from this study indicated that genotypes responded differently to Cu for grain yield, and for yield components. The expression and nature of deficiency symptoms varied among the genotypes. The first symptoms appeared over a period of time between 22 and 39 days after sowing, suggesting that there were differences among the test genotypes in sensitivity to Cu-deficiency. Among the six Cu-inefficient cultivars, Park, Roblin, and K. Tausi expressed deficiency symptoms earlier than Oslo, Katepwa, and Kwale. Kwale and Katepwa expressed symptoms much later, and their grain yields were significantly lower than that of Park. Stem and head melanosis, symptoms associated with Cu-deficiency, appeared on cultivars

Park and K. Tausi. This observation agreed well with the previous findings of Piening *et al.*, (1985) and Gartrell *et al.*, (1979). The stems that developed melanosis showed poor seed set and grain filling. In certain cultivars, expression of melanosis occurred on the glumes and rachis and, subsequently, on the grains (Graham and Nambiar, 1981). In this study, K. Leopard did not show deficiency symptoms on the vegetative parts, but showed grain discolouration, although it produced significantly higher grain yield than any of the other test cultivars on Cu deficient (-Cu) and Cu-sufficient (+Cu) soils. Relative degree of grain fill and discolouration are the characteristics which differentiated the sensitivity of K. Leopard and 5A/5RL wheat-rye translocated lines to Cu-deficiency. K. Tausi and Park, which were classified as sensitive to Cu-deficiency, showed melanosis on the grains. The relationship between melanism on grain and Cu-deficiency is not clearly understood although Piening and MacPherson (1985) have indicated that presence or absence of the bacterium *Pseudomonas cichorii.* is a confounding factor. Melanism on the stem and spike has been indirectly associated with the enhancement of Cu-inefficiency of the sensitive cultivars, partly by affecting the biochemical processes of photosynthesis which specially require Cu (Magdolna and Gabor, 1990). Stunted growth and excessive tillering, the symptoms that were noted on cv. Kwale, have been linked to the increase of the demand for Cu and immobilization of the limited Cu available in the plant system (Loughman *et al.*, 1983).

Copper concentrations in the young developing leaf tissues and organs decline with the progression of plant maturity (Loneragan, 1981), and Cu has been reported to be involved in mobilization of carbohydrate (Brown and Clark, 1977). Consequently, deficiency of Cu can affect the translocation and remobilization of carbohydrates, thereby contributing to the failure of grain formation and to poor grain filling. All 5A/5RL wheat-rye translocated lines showed Cu-efficiency in both the +Cu and -Cu treatments and did not have poor quality grains compared to cultivars lacking chromosome translocation. The deficiency symptoms, which included the withering of young leaves, necrosis and abortion of spikes on developing tillers, also appeared on the +Cu treatment on Katepwa, Kwale and K. Tausi. Similar expressions of the deficiency symptoms on wheat cv. Park grown in the field ameliorated with copper sulphate have been reported by Piening *et al*., (1985). This phenomenon, which varies with the genotype, could be related to the relatively low rate of movement of Cu within the plant systems (Clark, 1983) and implies an unsatisfied demand by the plant.

The movement of Cu within the various parts of the plant plays a vital role in the plant utilization of Cu. The capability of the root tissues of some wheat cultivars to limit Cu transported to the shoots under conditions of both Cu-deficiency and Cu-abundance (Loneragan, 1981) and differences in internal Cu requirements may have contributed to the deficiency symptoms on +Cu treatments on Kwale, Katepwa and K. Tausi. The appearance of symptoms on the youngest leaves, and failure of the production of spikes on Kwale, Oslo and Katepwa could be due to the relatively low rate of remobilization of Cu from senescent leaves and other plant organs to the newly growing tissues (Loneragan *et al*., 1980). Cultivars Biggar and Columbus did not show any symptom of Cu-deficiency although grain yield for cv. Biggar from the (-Cu) treatment was significantly lower than that of Columbus.

The analysis of variance (Table 5.0.0 and 5.1.0) for grain yield and yield components showed significant ($P \leq 0.01$) main effects due to translocation for grain yield, number of grains spike^{-1} and percent fertile florets spike^{-1}, except for the number of florets spike^{-1}. This illustrated the differences between the performance of the 5A/5RL wheat-rye translocated lines and the non-translocated wheat cultivars. The wheat-rye translocated lines produced high grain yield, grain number spike^{-1} and relatively higher percent fertile florets spike^{-1} from both +Cu and -Cu treatments for main stem and tillers. This contributed to the higher grain number spike^{-1} and grain yield pot^{-1}.

In this study, the effects of Cu were significant ($P \leq 0.05$) on all measured variables except for the number of florets spike^{-1} from tillers. This suggested that the number of florets spike^{-1} was not influenced by Cu status in the plant. However, in severe Cu-deficiency, the spikes abort, thereby affecting the entire organ (Graham and Nambiar, 1981). The cultivars that had relatively fewer spikes were Oslo, K. Tausi and Kwale. The Cu × translocation interaction was significant ($P \leq 0.05$) for grain yield and number of grains spike^{-1} for tillers (Table 5.0.0). These significant interactions illustrated differential response to Cu for grain yield between the 5A/5RL wheat-rye translocated lines and non-translocated wheat cultivars. Similarly, significant Cu x translocation interaction for grains spike from tillers indicated differential response to Cu for number of grains spike^{-1} between the wheat-rye translocated lines and wheat cultivars. Therefore, copper increased the number of grains produced spike^{-1} on tillers. Significant ($P \leq 0.05$) genotype × Cu interaction for grain yield illustrated differential response to Cu by the twelve genotypes tested.

The number of tillers plant^{-1} (including both seed-bearing spikes and the non-seed-bearing spikes) was significantly higher for CEG-2, K. Leopard, Kwale and Katepwa than for the other cultivars (Table 5.2.0). Of the four genotypes, CEG-2 and K. Leopard had productive tillers, while Kwale and Katepwa had tillers with no spike or malformed spikes. These findings are similar to those from the prior work of Smilde and Henkens (1967) and Nambiar, (1976), with different cultivars.

Copper efficiency is assessed by comparing the relative grain yield of a genotype grown in Cu-deficient conditions to the yield achieved with sufficient Cu supply. The genotype with the least Cu-induced yield loss has the highest Cu-efficiency (Graham *et al.*, 1987). The ability for CEG-2 and CEG-3 to produce three-fold grain yield more than the cv. Park on the Cu-deficient soil was attributed to the higher Cu-efficiency of 5A/5RL translocated lines (Table 5.3.0). The use of a efficiency index to determine efficient genotypes is of benefit to wheat breeders and farmers for several reasons. Firstly, the genotypes with the highest grain yield from Cu-deficient soils (-Cu) are of interest but they still respond to Cu applications, therefore, this index is often used to identify potential parents for breeding purposes. Secondly, the highest grain producers from Cu-deficient soils (-Cu) may be of immediate benefit to farmers. The 5A/5RL wheat-rye translocated lines were also consistently the highest yielders on the Cu sufficient soil, and had a relative grain production of 1.5 times higher than cv. Park in the pot experiments. This observation follows the definition of a Cu-efficient genotype as suggested by Graham (1984). These results also agreed well with the findings of the study conducted on 5RL/5BS, 5RL/4AS and 4BL/5RL near-isogenic lines, and their non translocated wheat parents, which indicated that the lines with 5RL wheat-rye chromatin were Cu-efficient (Graham *et al.*, 1981; Graham *et al.*, 1987; Podlesak *et al.*, 1990; Schlegel *et al.*, 1991). From the current study, it was evident that Cu-efficiency gene(s) on the chromosome 5RL offer some potential for exploitation by crossing the well adapted, but Cu-inefficient cultivars, to CEG-2, CEG-3 and CEG-5.

The separation of means showed significant differences in grain yield and yield components among the 5A/5RL wheat-rye translocated lines (Table 5.2.0). Lines CEG-2 and CEG-3 yielded significantly more grain than CEG-5, partly because of relatively lower percent fertile florets spike^{-1} and number of grains spike^{-1} for the main stem and tillers. Line CEG-2 had more productive number of tillers and well filled grains while CEG-3 had less tillers but had relatively larger grains.

High yielding wheat cultivars are required, but often they fail to produce to the maximum potential, partly because of a difficulty in satisfying their maximum nutritional requirements. If a deficiency arises, copper levels in the soil could be supplemented by fertilizer application. Among the three wheat cultivars which were bred in Kenya, K. Leopard was selected and tested before Cu treatments were used. However, the recently developed cultivars Kwale and K. Tausi were tested and selected under copper treatment regimes. Since diversity for Cu-efficiency exists within the wheat cultivars (Nambiar, 1976), the Cu-efficiency of K. Leopard could be associated with the environment under which it was developed, since it was selected indirectly on the basis of vigour and yield on

Cu-deficient soils. The Cu-inefficiency noted on the cvs. Kwale and K. Tausi may reflect the type of environment (Cu-treated regimes) under which these two cultivars were selected.

In comparing the Cu-efficiency among the six Canadian wheat cultivars, Columbus showed a relatively higher grain yield (1.5 times more) than cv. Park , and an efficiency of 76% (Table 5.3.0). Although the grain yield for cv. Biggar was comparable to cv. Columbus on the Cu-deficient soil, the efficiency (37%) was significantly lower. Low Cu-efficiency of cv. Biggar in this study could be due to limited volume of the soil in the pot as compared to field situation where roots could extensively grow. The cultivar Biggar produced higher grain yield on the Cu sufficient soil than cv. Columbus. Wheat-rye translocated lines were the highest in Cu-efficiency (70-127%) for grain yield probably due to the 5A/5RL wheat-rye translocated chromosome. However, there no significant differences between CEG-2 and CEG-3 for Cu-efficiency.

On the basis of grain yields, all the tested genotypes responded to Cu supply. Significant ($P \leq 0.01$) Cu effects on Kwale, K. Tausi and ($P \leq 0.05$) Biggar, Park, Oslo, Roblin and Katepwa indicated that these cultivars were Cu -inefficient. These inefficient cultivars showed low grain yield on Cu-deficient soil (-Cu), but produced higher grain yields on soils which had sufficient Cu (+Cu). The evidence from this investigation suggests that CEG-2, CEG-5, CEG-3, K. Leopard and Columbus are Cu- efficient although K. Leopard showed some grain appearance symptoms for Cu-deficiency.

5.4.0 CONCLUSION

Breeding wheats with improved Cu-efficiency is a worthwhile objective that can reduce Cu fertilizer requirements, and reduce grain yield losses from marginal deficiencies. From this study it was found that grain yield, grain number and percent fertile florets were the components that were affected by Cu-deficiency. Low fertility of florets and small shrivelled grains were mainly attributed to the deficiency of Cu.

Secondly, there were detectable variations for Cu-efficiency among the Canadian and Kenyan wheat cultivars. Columbus was found to be the most Cu-efficient of the six Canadian cultivars while K. Leopard was the most Cu-efficient among the Kenyan cultivars. Katepwa, Roblin, Oslo, K. Tausi and Kwale were sensitive to Cu-deficiency.

Thirdly, the study revealed that 5A/5RL wheat-rye translocated lines were Cu-efficient by producing high grain yield without showing deficiency symptoms on Cu-deficient soil. The efficiencies of the twelve genotypes therefore could be classified as follows: (i) Cu-inefficient genotypes which showed severe symptoms: Kwale, K. Tausi, Oslo, Katepwa and Roblin, (ii) Cu-inefficient genotypes which show no symptoms: K. Leopard and Biggar, and (iii) Cu-efficient genotypes with no symptoms showing: CEG-2, CEG-3 CEG-5 and Columbus. The evidence suggested that 5RL wheat-rye chromatin could be used as a source to incorporate Cu-efficiency into the Cu-inefficient cultivars and to improve their yielding ability on Cu-deficient soils. The study of segregating populations to detrmine the genetics nof the Cu-efficiency traits using 5RL sources yet remains to be done.

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SUMMARY

6.0.0 GENERAL DISCUSSION.

For the last three decades, increased productivity of wheat resulted from improved management and cultivars. Limited attention has been given to wheat improvement for mineral nutrient efficiency because fertilizers have been readily available. Copper deficient soils have been identified in Canada (Penney *et al* ., 1988; Karamanos *et al* ., 1986; Gupta and MacLeod, 1970), however Canadian wheat cultivars have not been characterized for Cu-efficiency prior to this study.

From the results of the field study, the eight test cultivars were found to be different in their Cu nutrition and Cu-efficiency at the Stony Plain site. The cultivar Biggar was found to be the most tolerant while cv. Oslo, Park, and Roblin were sensitive to Cu-deficiency in the field trial. The critical levels of Cu in the youngest fully emerged leaves of the eight test cultivars were found to vary with growth stage, cultivar and season, and this finding conformed with the reports of Bates (1971), and Graham and Nambiar, (1981). The critical Cu range in YFEL was between 2.3 and 5.7 $\mu g\ g^{-1}$ dry weight for cvs. Katepwa, Park, Oslo and Roblin. The efficiency of cv. Biggar was due to its apparent enhanced capability for Cu uptake and utilization. Cultivar Katepwa was found to be efficient in translocating Cu to the leaves but apparently has a high demand for Cu, as indicated by Cu deficiency symptoms expressed in the +Cu treatments. Cultivar Park had a high demand for Cu, possibly due to its high growth rate. This latter proposal follows the suggestion of Chapin (1987) that plants with high growth rates have high demands for nutrients. Copper in grains from main stems was relatively higher than in grains from tillers. The lowest mean Cu concentration in grains from tillers were found with cvs. Oslo, Park and Roblin (1.4-1.5 $\mu g\ g^{-1}$).

It was found that Cu concentrations in the flag leaves of the eight test cultivars were significantly correlated to the grain yield, the number of grains spike^{-1}, and percent floret fertility for cultivars grown on the -Cu treatment . However the range of Cu concentration in flag leaves was too small to be used as a method of detecting the Cu status of plants. From these findings, it could be speculated that Cu-efficiency of the tested cultivars depends mainly on the translocation of Cu to the young growing parts, and its further utilization in metabolism and growth.

From the dose response study under greenhouse conditions, it was found that the cultivar Biggar was relatively Cu-efficient while the cv. Oslo was sensitive to Cu-deficiency. The results indicated that the rate of 145 μg Cu kg^{-1} soil could be used to distinguish the efficient and inefficient cultivars on the Cu deficient soils from Stony Plain, in a bioassay conducted in the greenhouse.

The genetic variability for Cu-efficiency among the test cultivars is low, and introduction of genes for Cu-efficiency from wheat-related species could therefore be of importance. From this study, it was found that the 5A/5RL wheat-rye translocated lines were Cu-efficient under greenhouse conditions. These findings align with the reports by Graham *et al.* (1987), Podlesak *et al.* (1990), and Schlegel *et al.* (1991) that 5RL/5BS, 5RL/4BS, 5RL/4AS, 5RL/5BS, and 5BL/5RL wheat-rye translocated lines are Cu-efficient. Although a greenhouse study was used for screening wheat lines and cultivars, the final appraisal for Cu-efficiency of cultivars should be conducted on Cu-deficient soils in the field.

6.1.0 CONCLUSION.

Firstly from the field and greenhouse study, cv. Biggar was found to be relatively Cu-efficient , while cv. Park Oslo and Roblin were found to be the most sensitive to Cu-deficiency in the soil. Secondly, there is a need to apply Cu to Stony Plain soil as a short term solutions in order to alleviate Cu-deficiency problem. Thirdly, 5A/5RL wheat rye translocated lines proved to be Cu-efficient and this could be used as a source of genetic variability for the breeding of Cu-efficiency for Canadian and other cultivars, although the mode of inheritance of the trait from the 5A/5RL is not yet known

6.2.0 FUTURE RESEARCH.

Further research into the efficiency of 5A/5RL wheat-rye translocated lines for Cu is strongly suggested. One of the areas that should be further investigated, is the mechanism of Cu-efficiency, particularly concerning the physical and chemical behaviour of roots in Cu-deficient soils. There is an urgent need to elucidate how Cu-efficiency is conferred by further determination of the type, amount, and reactions of the root exudates from efficient and inefficient cultivars in +Cu and -Cu media. This may lead to the development of a method of identifying sensitive and tolerant wheat cultivars to Cu-deficiency. Another important area to study regarding Cu-efficiency could involve the development of 5A/5RL near-isogenic lines, to assist in determining the mode of genetic control. Cultivars Biggar, Park, Oslo, Katepwa, K. Tausi, Kwale (sensitive cultivars) and K. Leopard be backcrossed (BC_5 F_1) to CEG-2, CEG-3 and CEG-5. Near-isogenic lines should be selected using 'hairy peduncle' as a genetic marker to confirm the presence of 5RL chromatin. The isogenic lines would then be tested on paired Cu treatment plots in the field in areas with Cu- deficient soils. Lastly, it would be desirable to conduct a genetic study to find how many genes are involved in Cu-efficiency.

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APPENDIX

Appendix 2.0.0. Analysis of variance for percent copper efficiency use for grain yield of eight Canadian spring wheat cultivars, grown at Stony Plain, Central Alberta. (Data were Transformed using arcsine).

Source	df	Mean squares % Cu-efficiency
Rep	3	187.531
Cultivar	7	486.001*
Year	1	3282.000**
Cultivar × Year	7	470.393*
Error	45	171.740
cv (%)		20.36

*, ** significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.

Appendix 2.1.0. The effect of copper on the number of grains spike^{-1} on the main stems, and tillers and grain yield of eight Canadian spring wheat cultivars grown in 1990 and 1991 at Stony Plain.

	Number of grains spike^{-1}								Mean grain yield g plant^{-1}			
	Main stems				Tillers							
	1990		1991		1990		1991		1990		1991	
Cultivar	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu
Katepwa	30.8	34.3	31.7	39.7	26.2	29.1	25.9	33.5	7.3	7.5	4.6	6.2*
Roblin	26.6	30.0	28.5	33.5*	15.4	21.9	22.3	28.4	3.1	3.5	3.2	4.5**
Park	25.6	31.4	27.9	31.3*	14.3	24.2*	19.8	23.8	3.0	4.0	2.8	3.4**
Laura	35.1	32.4	24.7	41.9*	20.0	20.4	17.8	29.1*	3.6	3.3	2.1	4.5**
Conway	31.2	27.8*	25.1	33.0**	23.4	23.8	18.9	26.7**	5.9	4.6	2.6	4.9*
Oslo	28.1	33.0	25.8	42.9**	16.7	24.4	18.6	32.9*	3.0	4.4*	2.4	5.1**
Columbus	23.7	23.0	27.8	32.3	18.8	18.6	24.7	27.7	4.0	4.2	3.6	5.1
Biggar	51.5	53.5	49.5	48.3	36.2	36.9	38.8	38.4	7.5	6.2	5.7	5.9

*, ** - significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.

Appendix 3.0.0 Mean concentrations of copper ($\mu g\ g^{-1}$ dry weight) in the yongest full emerged leaves (YFEL) of eight Canada spring wheat (*T. aestivum* L.).

		Cultivar							
DAS (Year)		Katepwa	Roblin	Park	Laura	Conway	Oslo	Columbus	Biggar
21 (1990)	-Cu	7.5 ± 0.7	7.5 ± 0.5	7.2 ± 0.5	7.4 ± 0.4	8.4 ± 0.5	8.5 ± 0.5	8.5 ± 0.3	7.6 ± 0.4
	+Cu	10.8 ±0.6	8.6 ± 0.7	7.7 ± 0.5	8.1 ± 0.3	7.9 ± 0.5	9.5 ± 0.5	9.2 ± 0.4	8.3 ± 0.1
(1991)	-Cu	5.0 ± 0.6	5.1 ± 1.2	3.6 ± 0.2	3.2 ± 0.4	3.9 ± 0.2	3.6 ± 0.3	4.2 ± 0.3	4.4 ± 0.2
	+Cu	5.5 ± 0.5	4.1 ± 0.2	4.8 ± 0.2	4.3 ± 0.2	6.7 ± 0.8	4.7 ± 0.3	4.7 ± 0.7	4.9 ± 0.3
28 (1990)	-Cu	5.7 ± 0.3	4.6 ± 0.1	4.8 ± 0.2	4.7 ± 0.2	5.2 ± 0.3	4.6 ± 0.1	5.4 ± 0.2	4.7 ± 0.3
	+Cu	6.4 ± 0.6	5.2 ± 0.5	5.6 ± 0.4	5.8 ± 0.4	5.9 ± 0.3	5.9 ± 0.2	6.3 ± 0.2	5.9 ± 0.6
(1991)	-Cu	3.5 ± 0.6	2.8 ± 0.3	3.1 ± 0.1	3.1 ± 0.2	2.3 ± 0.5	2.3 ± 0.4	2.6 ± 0.4	2.5 ± 0.4
	+Cu	4.8 ± 0.4	3.7 ± 0.3	4.3 ± 0.3	3.5 ± 0.4	3.7 ± 0.5	3.8 ± 0.4	4.6 ± 1.1	4.1 ± 0.3
35 (1990)	-Cu	4.2 ± 0.4	4.1 ± 0.7	3.5 ± 0.4	3.7 ± 0.3	4.4 ± 0.5	3.5 ± 0.2	4.1 ± 0.3	3.7 ± 0.4
	+Cu	6.4 ± 0.9	4.5 ± 0.6	4.4 ± 0.4	4.5 ± 0.4	4.4 ± 0.3	4.4 ± 0.3	4.5 ± 0.3	5.2 ± 0.6
(1991)	-Cu	2.7 ± 0.4	2.7 ± 0.1	2.6 ± 0.5	2.4 ± 0.2	2.4 ± 0.4	2.3 ± 0.3	2.6 ± 0.4	2.4 ± 0.3
	+Cu	4.5 ± 0.2	3.1 ± 0.1	3.8 ± 0.3	2.5 ± 0.4	3.4 ± 0.3	3.2 ± 0.3	3.2 ± 0.5	3.7 ± 0.3
42 (1990)	-Cu	3.6 ± 0.3	2.7 ± 0.3	2.9 ± 0.3	3.1 ± 0.3	3.4 ± 0.2	2.6 ± 0.3	2.9 ± 0.4	3.1 ± 0.5
	+Cu	4.4 ± 0.6	3.1 ± 0.3	3.2 ± 0.4	3.3 ± 0.1	4.1 ± 0.3	2.7 ± 0.2	3.7 ± 0.2	3.7 ± 0.2
(1991)	-Cu	2.2 ± 0.2	1.5 ± 0.2	2.0 ± 0.3	1.8 ± 0.2	2.6 ± 0.3	1.5 ± 0.1	2.3 ± 0.6	1.9 ± 0.1
	+Cu	3.4 ± 0.4	2.6 ± 0.1	2.8 ± 0.2	2.0 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	2.6 ± 0.3	2.5 ± 0.2
51 (1990)	-Cu	2.9 ± 0.2	2.5 ± 0.5	1.9 ± 0.3	2.7 ± 0.5	2.4 ± 0.2	1.7 ± 0.3	2.7 ± 0.4	3.8 ± 1.0
	+Cu	4.1 ± 0.8	2.3 ± 0.4	2.5 ± 0.3	2.9 ± 0.3	3.1 ± 0.3	2.5 ± 0.4	2.7 ± 0.2	4.0 ± 0.7
(1991)	-Cu	1.8 ± 0.4	1.6 ± 0.3	1.4 ± 0.1	1.3 ± 0.3	1.5 ± 0.1	1.3 ± 0.1	1.7 ± 0.1	1.8 ± 0.5
	+Cu	2.3 ± 0.1	1.9 ± 0.4	2.4 ± 0.2	2.2 ± 0.1	2.0 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	2.2 ± 0.2

Values are means of four replicates ± standard errors.

Appendix 3.1.0. Mean squares for Cu concentrations in the youngest fully emerged leaves of eight Canadian spring wheat cultivars, grown at Stony Plain, Central, Alberta.

		Days aftter sowing									
		21		28		35		42		51	
Source	df	1990	1991	1990	1991	1990	1991	1990	1991	1990	1991
Rep	3	9.678	0.636	2.360	4.807	6.743	2.270	2.970	0.265	7.621	0.195
Cultivar	7	3.234**	2.267	1.090*	1.192	1.490**	0.878	1.827**	0.883*	3.319**	0.191
Cultivar × Rep (E_a)	21	0.494	1.132	0.434	0.771	0.304	0.395	0.347	0.341	0.665	0.251
Cu	1	15.000**	11.902**	13.230**	25.882**	11.306**	12.960**	3.950**	6.375**	3.018**	5.118**
Cultivar × Cu	7	1.833**	2.160	0.114	0.589	0.954	0.598	0.151	0.367	0.352	0.139
Error	21	0.338	0.993	0.270	0.573	0.924	0.354	0.221	0.273	0.398	0.279
cv (%)		7.06	22.02	9.61	22.11	22.12	20.10	14.46	22.88	22.79	29.34

*, ** significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.
Ea - Error for cultivar.

Appendix 5.0.0 The effect of copper on the plant height and number of tillers plant^{-1}

Genotype	Plant Height (cm)		Tillers plant^{-1}	
	-Cu[§]	+Cu[§]	-Cu	+Cu
CEG-5¶	65.5	58.2	5.0	5.0
CEG-2¶	61.5	63.9	6.0	7.0
CEG-3¶	78.3	76.3	4.0	5.0
K. Leopard	66.8	66.7	7.0	7.0
K. Tausi	57.4	66.0	6.0	5.0
Kwale	39.7	45.2	6.0	8.0
Oslo	53.7	53.2	4.0	5.0
Katepwa	51.3	59.2	6.0	6.0
Roblin	50.2	50.8	5.0	4.0
Columbus	59.1	60.4	6.0	6.0
Park	58.9	63.1	5.3	5.6
Biggar	51.9	54.9	5.0	5.0

¶ - 5A/5RL wheat-rye translocated lines.
§ - Means of four replicates.

Appendix 5.1.0. Analysis of variance for percent grain yield copper efficiency use of twelve wheat genotypes, grown under greenhouse conditions. (Data were transformed using arc sine).

Source	df	Mean squares % Cu-efficiency
Rep	3	45.647
Cultivar	11	1880.027**
Error	33	219.547
cv (%)		33.9

*, ** significant ($P \leq 0.05$) and ($P \leq 0.01$), respectively.